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CONTENTS

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INVESTIGATIONS ON SWEETCLOVER FAILURE IN SOUTHWESTERN ONTARIO[1]

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INTRODUCTION

Although in southwestern Ontario the sweetclover crop is highly valued for soil improvement, forage, and bee pasture, farmers in that area are gradually abandoning its use because of the increasing incidence of "failure". In recent years unprofitable stands have been alarmingly frequent with the commonly grown biennial varieties of the white blossomed species, *Melilotus alba* Desv., and also with the variety Common Yellow of the yellow blossomed species, *M. officinalis* (L.) Lam., in nearly every area where sweetclover has been extensively grown.

An intensive investigation of the causes of failure in biennial sweetclover was conducted during the period from June, 1947, to June, 1949. Early in the investigation it became apparent that in certain instances a major loss of stand occurred during the second year. In some fields the stand deteriorated in both the first and second seasons. As more than one factor, operating at different stages of the plant growth, appeared to be responsible for loss of stand, it became necessary to examine fields repeatedly during successive seasons to discover the cause of the damage and the time of its occurrence.

Unfavourable weather and cultural conditions, which are among the first factors commonly considered when crop loss occurs, were primarily responsible for some of the failures. Prolonged drought shortly after the seed germinated and excessively heavy or lodged nurse crops has resulted in certain seedling losses. Frost heaving during the winter and early spring also caused severe losses in some instances in overwintering crops. Frequent examination of sweetclover roots indicated that nodulation was generally good and minimized the possibility that any of the failures were the direct result of a deficiency of legume bacteria.

Since it is generally recognized that sweetclover cannot be grown successfully if the calcium content of the soil is very low, considerable attention was paid to chemical analysis of soil samples[3] collected from many sweetclover fields. In addition to a determination of their calcium content and pH, all samples were analysed for available phosphate, nitrate, and potash, and some for magnesium and organic matter as well. These analyses

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[3] Soil analyses were performed by the Dominion Experiment Station, Harrow, Ontario, and the Department of Soils, Ontario Agricultural College, Guelph, Ontario.

showed a positive correlation between poor stands of sweetclover and low calcium in some fields, especially on sandy soils. In some of the latter fields, there were patches in which the seedlings were severely stunted, with weakly developed roots bearing few or no nodules. Most of these seedlings disappeared quickly in hot, dry weather. The calcium content of such areas was extremely low, while in other areas of the same fields, where the stand was thicker and more vigorous, the calcium content was much higher. Other fields were examined in which calcium was generally low, but no definite correlation could be found between poor seedling survival and low calcium content. Other minerals were also deficient in certain fields, and generally when calcium and pH were low, phosphate, nitrate, and potash were also low, but no consistent correlation could be shown between severe deficiencies of the latter minerals and poor stands.

Insect pests also merited consideration as a cause of sweetclover failure. The sweetclover weevil, *Sitona cylindricollis* Fahr., which was described by Bird (1) as a seriously destructive pest in Manitoba, was widely distributed. Its feeding was evident in nearly every field observed, but it was a major cause of plant loss in only a few fields. During the course of the current investigation, a total of four fields were observed to have been severely damaged by weevils. Two of these were at the seedling stage and the other two were overwintered crops. In all 4 fields large numbers of the plants died. Thus, it is possible that the sweetclover weevil has been the cause of certain of the failures of sweetclover in past years in southwestern Ontario.

As indicated above, weather conditions, lime deficiency, and insect pests have caused considerable losses in stands of sweetclover, but the most widespread and serious damage observed in Essex and Kent counties appeared to be caused by rootrots. A number of pathogenic fungi, including *Phytophthora cactorum* (Leb. and Cohn.) Shroet, *Fusarium* spp., *Rhizoctonia solani* Kuehn, *Sclerotinia trifoliorum* Eriks., *Mycosphaerella lethalis* Stone*, *Ascochyta imperfecta* Pk.*, and *Cylindrocarpon sp.*, were isolated frequently from diseased sweetclover roots. Of these, *P. cactorum* proved to be most widely distributed and destructive, and hence it was subjected to the investigations outlined below. Seedling blight caused by various fungi was also destructive, and was investigated to a more limited extent. Other diseases, including mosaic, black stem, and Pseudopeziza leaf spot, were commonly observed, but they appeared to be of minor importance in reducing stands, and thus received scant consideration in the present study.

PHYTOPHTHORA ROOTROT

Preliminary investigations definitely indicated that Phytophthora rootrot was one of the important factors involved in sweetclover failure in Essex county. This disease, which has undoubtedly been present in Essex country for many years, was found in 25 of the 26 fields examined in June, 1947, when this investigation was begun. During the spring of 1948 it was found in 23 of 27 fields, and during 1949 in 48 of the 52 fields inspected. It appeared to be responsible for the loss of more than 30 per cent of the plants in at least one-third of the fields in Essex county during each of the

* Identified by F. R. Jones, Senior Pathologist, Division of Forage Crops and Diseases, U.S.D.A., Department of Pathology, University of Wisconsin, Madison, Wisconsin.

3 years, and certain fields were found in which more than 75 per cent of the plants were apparently killed by this rootrot disease. It was also indicated to be a major hazard in Kent county, but was generally less severe there than in Essex county.

In 1939, Jones (3) reported a rootrot of sweetclover caused by Phytophthora in Ohio, Indiana, Illinois, and other parts of north central United States. In 1940, Cormack (2) reported a similar rootrot in Alberta, Canada, caused by *Phytophthora cactorum* (Leb. and Cohn.) Shroet. The disease herein described is apparently identical with that reported by Jones and by Cormack. Cultures of *P. cactorum* obtained by Cormack in Alberta were indistinguishable in morphology and pathology from cultures of the fungus isolated from sweetclover in Ontario.

SYMPTOMS

The first evidence of Phytophthora rootrot in Ontario may be found in certain first year stands of sweetclover in the fall before the leaves have become frozen. At this time a few wilted plants with root lesions typical of the disease may sometimes be found. However, in Essex county, relatively few diseased plants may be found until the latter half of April when rootrot suddenly becomes prevalent at a time when the plants are in a state of active vegetative growth. Although the soil is usually cool and moist at this time, many plants may be found in a wilted condition. When the roots are dug, soft decayed regions may be found on any part of the primary roots and often on secondary roots as well (Figure 1). If a decay develops in the primary root near the crown, the above-ground portions of the plant wilt and may soon die. Characteristic, non-septate hyphae can easily be observed microscopically in thin, unstained sections of tissue from the margin of diseased portions of the root. When the soil is wet and cool, the diseased regions may be only slightly discoloured although soft and watery and heavily populated with bacteria. As the season advances and the soil becomes dry, diseased areas become shrunken and brown in colour. Many weakly parasitic and saprophytic organisms soon invade the diseased roots, and the root symptoms then become varied. Although the disease is most active in late April and early May in southwestern Ontario, plants affected by Phytophthora rootrot may be found wilting and dying even until mid-summer.

ISOLATION AND CULTURE

Roots with incipient lesions were selected for isolation purposes, but even these were heavily colonized with bacteria, thus making isolation somewhat difficult. Such root portions were washed, then surface-sterilized with mercuric chloride solution. Small pieces of tissue were cut from the margin of the lesions, planted on potato dextrose agar, and then incubated at 10° to 15° C. It was found that bacteria could often be eliminated by cutting the agar in a Petri dish into 8 sectors by means of a flamed metal spatula. Portions of infected root tissue were planted on alternate sectors, after which the neighbouring sector was placed on top, thus sandwiching the root tissue between 2 layers of agar medium. Phytophthora hyphae which penetrated the agar could then be transferred to a test tube slant.

Potato dextrose agar was found to be highly suitable for vegetative growth and oöspore production, but the fungus also grew satisfactorily on cornmeal and oatmeal agars. Media prepared from steamed cornmeal, cornmeal-sand mixtures, wheat bran, barley bran, pieces of sweetclover, and various mixtures of cornmeal, peas, and sand proved to be more or less unsatisfactory. Optimum growth of the fungus was obtained on steamed, sliced potatoes and on cooked, rolled oats, either whole or with hulls removed. On the latter medium, prepared by moistening a thin layer of rolled oats in the bottom of a small Erlenmeyer flask and sterilizing in an autoclave, the fungus grew exceptionally well, producing a thick mat of mycelium with an abundance of oöspores. This mat was then removed from the flask, washed free from excess rolled oats, and cut into small pieces either for the inoculation of roots, or for the infestation of soil.

INFECTION EXPERIMENTS

Inoculum of *P. cactorum* for use in infection experiments consisted either of cultures on potato dextrose agar or the mycelial mat produced by growing the fungus on rolled oats. Arctic or Commercial Yellow sweetclover plants of various ages, which had been transplanted into pots in the greenhouse were inoculated by placing the inoculum in contact with the roots, about 2 inches below the crown. In some experiments the inoculum was merely placed in contact with the unwounded root, but after it was found that a higher percentage of plants became infected when the inoculum was placed in a wound, the latter procedure was followed in later experiments. In most greenhouse experiments the inoculated plants were kept in moist soil at a temperature of about 18° C. Under these conditions 60 to 90 per cent of the inoculated plants of various ages usually became infected and wilted within 10 days.

In some experiments plants were not infected artificially, but were merely transplanted into soil collected from fields where rootrot was severe. Up to 50 per cent rootrot was attained in this way when temperatures were favourable.

In a number of experiments seedling infection by *P. cactorum* was tested by sowing sweetclover seed in soil infested with a culture of the fungus on potato dextrose agar, or the finely-minced, mycelial mat from a rolled oat culture of the fungus. When seedlings were grown in sterilized soil infested in this manner the percentage emergence was almost as high as in non-infested soil, but most of the seedlings "damped-off" within a week after emergence. Hyphae and oöspores of *P. cactorum* could be observed microscopically in the diseased seedlings, and pure cultures of the fungus could be recovered from them. Additional experiments showed that seedlings of the 9 varieties of *M. alba*, 4 of *M. officinalis*, and 1 of *M. suaveolens* tested were also susceptible to this type of post-emergence blight in artificially infested, sterilized soil. Despite the complete susceptibility of sweetclover seedlings to *P. cactorum* under the artificial conditions outlined above, evidence of seedling blight attributable to *P. cactorum* was not obtained in any untreated field soils either in the field or in the greenhouse experiments. Attempts to induce seedling blight by adding inoculum of *P. cactorum* to unsterilized field soils proved inconsistent because of other complicating factors. This problem is discussed below under "Seedling Blight of Sweetclover".

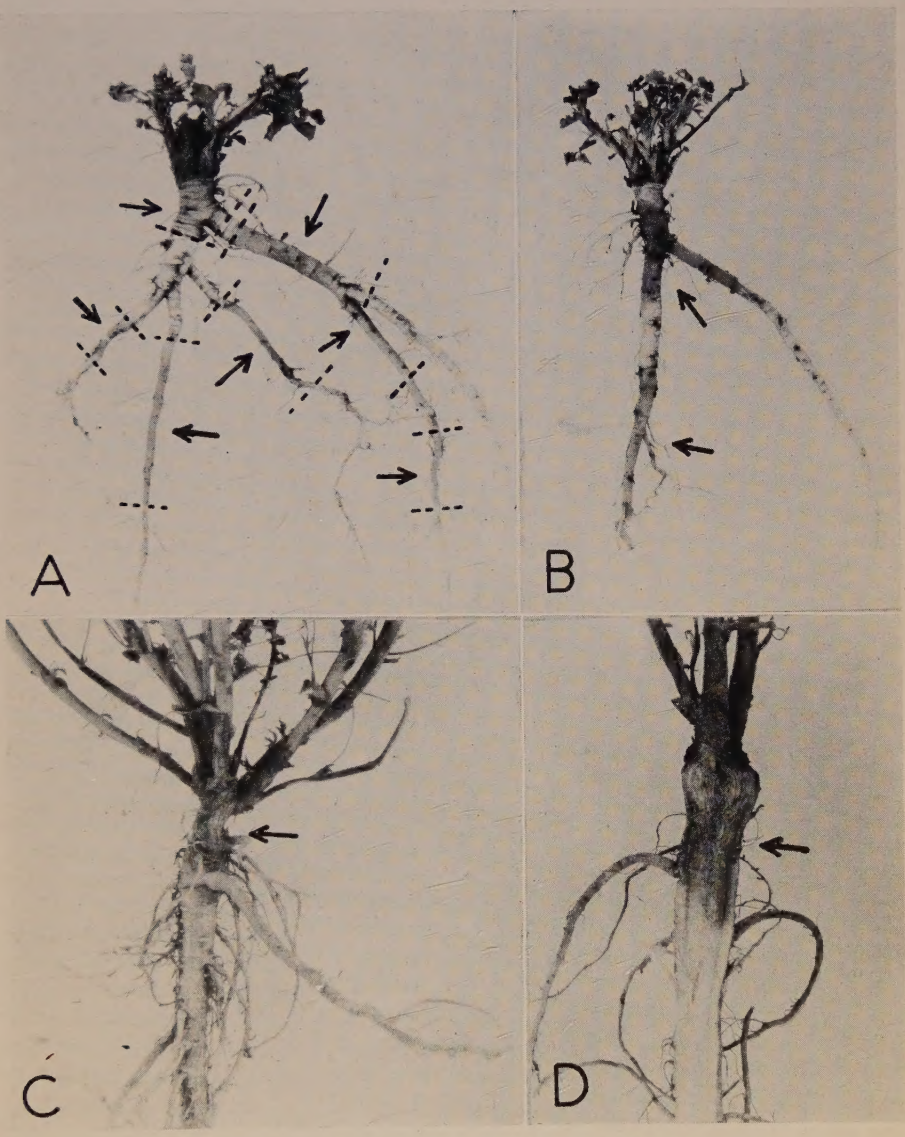

FIGURE 1. Phytophthora rootrot of sweetclover. A and B—roots collected in mid-April. Note the slight discolouration in A, and the occurrence of lesions not only on the main root but also at various locations on branch roots. C—root collected late in May. Note the shrunken lesion just below the crown. D—root split longitudinally showing internal discolouration of tissues in region of lesion.

Factors Influencing Rootrot Severity

A characteristic of sweetclover rootrot caused by *P. cactorum* is the manner of its development. Where it occurs it is sometimes observed in mild form in first year stands during October, after which it suddenly appears in the same fields the following April in its more destructive form. This phenomenon suggested that either the stage of host maturity or some seasonal climatic factor (or factors) strongly influences disease development. Accordingly, a study was made of some factors that may influence the seasonal occurrence of this disease. The relation of other factors to rootrot development was also investigated.

Susceptibility of Sweetclover Plants of Various Ages to P. cactorum

A test was made of the relative susceptibility of sweetclover plants of different ages to Phytophthora rootrot. Plants of the Commercial Yellow variety were planted in 10-inch pots. Three-quarters of those in each age group were inoculated with *P. cactorum* by placing mycelium against the roots; the rest were retained as checks. Results of this experiment, 3 weeks after inoculation, are given in Table 1.

TABLE 1.—SUSCEPTIBILITY OF SWEETCLOVER ROOTS OF VARIOUS AGES TO INFECTION BY *P. cactorum*

Age and condition of plants	Checks		Inoculated	
	Number of plants	Per cent of infection	Number of plants	Per cent of infection
Three weeks	20	0	60	60.0
Two weeks	14	0	42	33.3
Six months	12	0	36	80.5
Six months (frozen 3 months)	12	0	36	63.9
Eight months (frozen 3 months)	12	0	36	83.3
Ten months (frozen 3 months)	8	0	24	70.8

Table 1 indicates that inoculated plants of all ages from 3 weeks to 10 months readily became infected with Phytophthora rootrot. Somewhat less infection occurred in the plants 2 months old than in the others, but even one-third of them were killed. The plants 6 months of age that had been subjected to freezing temperatures for 3 months were not quite so severely infected as plants of the same age which had not been frozen; hence the freezing action apparently did not increase the susceptibility of the plants to infection. The results of this experiment, therefore, did not indicate that the age of the plants is of any particular significance in regard to the extreme severity of *P. cactorum* rootrot in the spring of the year.

The Relation of Temperature to P. cactorum Rootrot

In view of the lack of specific information concerning the relation of soil temperature to rootrot of sweetclover caused by *P. cactorum* and the distinct possibility that this factor might be primarily responsible for the phenomenal activity of the disease in the early spring, investigations of this phase were undertaken. Nevertheless, a low optimum temperature

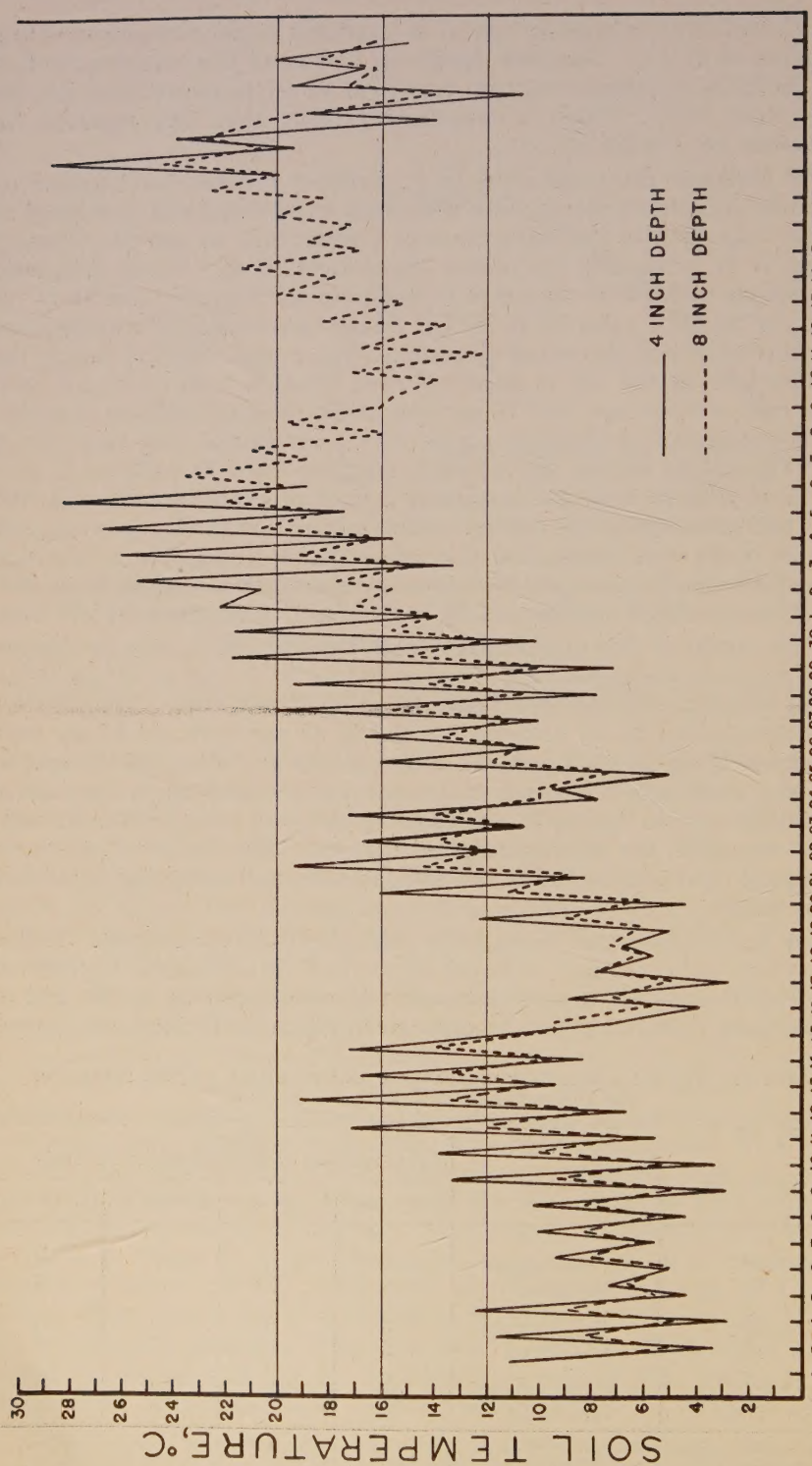

FIGURE 3. Soil temperatures at 4 inches and 8 inches below the surface during April and May, 1949. Readings taken at 8.00 a.m. and 6.00 p.m. daily at Harrow, Ontario.

with prevailing soil temperatures, the temperature readings 4 and 8 inches below the surface of the soil were recorded at 8.00 a.m. and 6.00 p.m. daily at Harrow during April and May, 1949. The readings are shown graphically in Figure 3. They show that the temperature of the soil surrounding the roots was considerably above freezing, i.e., the mean of the 2 daily readings was at least 6° C. at the 4- and 8-inch levels, before severe rootrot became evident. On the other hand, most of the destruction was already apparent even before the highest temperature readings had reached 20° C. at the 4-inch depth and 15° C. at the 8-inch depth. The temperature at the 4-inch depth exceeded 16° C. at 6.00 p.m. on only 11 days in April, but, since the 8.00 a.m. readings were always 6.6° to 12.2° C. lower, the temperatures could not have been even as high as 16° C. for more than a few hours each day. It seems evident, therefore, that *P. cactorum* rootrot of sweetclover is most destructive when the soil around the roots is somewhere between 6° C. and 16° C.

All of the data so far obtained on the relation between temperature and *P. cactorum* rootrot of sweetclover indicate that the temperature of the soil has a critical influence on the incidence of rootrot. Low soil temperatures like those prevailing in southwestern Ontario in April favour the vegetative growth and pathogenic activity of the *P. cactorum* causing rootrot of sweetclover.

The Effect of Moisture on the Severity of Rootrot

While inspecting fields for Phytophthora rootrot, the author occasionally observed that rootrot was most severe in the low, wet parts of the fields, and in dead furrows. Occasionally, when severe rootrot was found throughout an entire field, it was quite evident that the moisture was excessive in the entire field for a considerable period during the spring. These observations indicated that excessive soil moisture favours rootrot development. On the other hand, severe rootrot has been found on well drained soil, and, in some fields where the disease occurred, it was just as severe directly above the tile drains as in other parts of the field where drainage was poor.

In the soil temperature experiment described above, 4 levels of soil moisture were maintained at each temperature. The root infection resulting from artificial inoculation with *P. cactorum* was greater at the lower moisture levels, 35 per cent to 45 per cent M.H.C. (Table 2). These results were contrary to the observations of the occurrence and development of the disease in the field. It is highly probable, however, that the influence of moisture would be minimized when the inoculum was placed against the root as was the case in the controlled experiment. Other experiments on the relation of moisture to rootrot were attempted in naturally infested field soil. No significant differences in rootrot were obtained between wet and dry soils, but, since relatively low rates of infection were obtained under the relatively high temperatures that prevailed during the experiment, the results cannot be considered conclusive.

Influence of Soil Type on the Incidence of Rootrot

In a survey of 72 sweetclover fields in Essex and Kent counties in 1949, rootrot was found in 57 of them. As shown in Table 3, rootrot was present in all fields with a heavy type of soil (heavy loam to clay) and severe

TABLE 3.—THE OCCURRENCE AND SEVERITY OF PHYTOPHTHORA ROOTROT ON LIGHT AND HEAVY SOILS

Soil texture	Fields inspected	Number of fields showing			
		0 per cent	Trace to 10 per cent	10 to 50 per cent	Over 50 per cent
Heavy loam to clay	49	0	22	16	11
Sand to sandy loam	23	15	8	0	0

damage occurred in most of them. Less than one-third of the fields with sand to sandy loam soils showed any rootrot, and even when the disease occurred on these soils it was on no occasion very destructive.

Twenty-six fields were inspected in Oxford, Brant, Dufferin, Huron, and Bruce counties in May, 1949. Phytophthora rootrot was found in 3 of the fields, 2 of which were clay, and the other loam. Nineteen of the fields free from rootrot contained sand to sandy loam soils, 3 loam, and 1 clay.

On the basis of the above observations, it appears that the physical characteristics of the soil may have a significant influence on rootrot severity. Several experiments were, therefore, conducted in the greenhouse to test further the relation of soil texture to rootrot severity. Plants growing in clay and sandy soils were artificially inoculated with *P. cactorum* by placing inoculum in contact with the roots. A high percentage of rootrot developed in both the clay and sandy soils, with no significant difference between the two soil types. However, the artificial method of inoculating the roots by placing the inoculum in contact with the root possibly eliminated any influence soil texture may have had on root infection.

An experiment was begun in August, 1948, to compare the relative capacities of different types of naturally infested soil to induce rootrot. Clay soil was collected from a field in which Phytophthora rootrot had been severe during the current year. Sand and loam soils were collected by a random sampling method from 2 different areas in another sweetclover field. Rootrot had occurred on sweetclover in the latter field, but had been more severe on the loam than on the sandy soil. Four 10-inch pots were filled with each soil and six 4-months-old sweetclover plants were transplanted into each of them. The potted plants were exposed to outdoor conditions until the end of December, when they were slowly thawed, then placed in a greenhouse at approximately 18° C. to encourage infection. After 10 weeks the plants were examined for Phytophthora rootrot, and the results from this first planting were recorded (Table 4). The diseased roots were then cut into small portions and mixed with the soil in which they were grown. Five weeks later, 15 sweetclover plants were transplanted into each pot of soil and placed in a greenhouse where they were kept at 18° C. for 5 weeks. The percentage of rootrot occurring in the different soils in this second planting was then determined (Table 4).

As shown in Table 4 severe rootrot occurred on sweetclover planted in all 3 naturally infected soils, but in both plantings it was less than half as severe in the sandy soil as in the loam and clay. Further experimentation would be necessary, however, to determine whether, with repeated cropping, rootrot would become equally severe in all 3 types of soil.

TABLE 4.—INCIDENCE OF ROOTROT IN NATURALLY INFECTED SOILS OF DIFFERENT TYPE

	Percentage rootrot	
	First planting	Second planting
Clay	45.8[1]	36.6[2]
Loam	41.7	36.6
Sand	16.6	15.0

[1] Percentage rootrot among 24 plants.
[2] Percentage rootrot among 60 plants.

Crop Sequence in Relation to Rootrot

In order to determine whether cropping practice influences the severity of rootrot, information was obtained on the crop history of some of the fields in which sweetclover was growing. A special attempt was made to find any possible correlation between the frequency of sweetclover crops and the severity of rootrot. On the basis of the information obtained, it was indicated that the severity of rootrot was related to the frequency of sweetclover crops in the fields concerned. On the other hand, severe rootrot damage also occurred in certain fields in which sweetclover had been grown only once in 6 years.

A preliminary greenhouse experiment was conducted to determine the effect of preceding crops of sweetclover, alfalfa, red clover, soybeans, wheat, and corn on the incidence of Phytophthora rootrot in infested soils. This experiment indicated that rootrot was almost equally severe following some of the other legumes as it was after sweetclover, but it was less severe after wheat. Further experiments are necessary to ascertain the importance of crop sequence on Phytophthora rootrot, but the present evidence indicates that intensive investigations of this phase of the rootrot problem may be of value.

SEEDLING BLIGHT OF SWEETCLOVER

As previously indicated, seedling blight has been another important phase of sweetclover failure in southwestern Ontario. This type of failure is difficult to detect and evaluate by ordinary field inspection, partly because it is difficult to locate farmers' fields for inspection at the correct time to detect diseased seedlings, and partly because much of the seedling blight of sweetclover is of a pre-emergence type, which cannot be diagnosed in the field without finding the diseased, sprouted seeds in the soil.

Tests for seedling blight in field soils consisted of sowing disease-free sweetclover seed in steamed and non-steamed samples of the suspected soil. In some soils the emergence and survival of seedlings was as high in the non-steamed as in the steamed portions. Other soils were tested, however, in which the emergence was at least 20 per cent lower in the non-steamed than in the steamed portions, and some of the seedlings developed lesions or "damped-off" after emergence.

Many fungi were isolated from diseased sweetclover seedlings that had been surface-sterilized and planted on acidified and non-acidified potato dextrose agar. The various isolates were tested for pathogenicity on sweetclover seedlings by first culturing them on 5 per cent cornmeal-sand

medium in small flasks for 2 to 3 weeks, then infesting steamed soil in 4-inch or 5-inch pots with this inoculum, and sowing 50 or 100 disease-free seeds of the Commercial Yellow variety in each pot of soil. Pathogenicity was judged by comparing the emergence of seedlings in the infested soil with the emergence in the non-infested soil, and also by noting the post-emergence blight. Fungi that proved pathogenic in these preliminary tests were usually re-tested in both steamed and non-steamed soil.

The pathogenic fungi most frequently isolated from diseased sweetclover seedlings grown in various field soils included isolates of the following in descending order of occurrence: *Fusarium spp.*, *Pythium ultimum* Trow, *P. debaryanum* Hesse, and *Rhizoctonia solani* Kuehn. Although many of the *Fusarium* isolates were non-pathogenic, mildly to strongly pathogenic forms were also frequently obtained. The latter caused pre-emergence blight varying in incidence from a trace to 100 per cent and in addition caused post-emergence blight in both steamed and non-steamed soil. The *Pythium spp.* also caused a high percentage of pre-emergence blight as well as considerable "damping off" after emergence in both steamed and non-steamed soil. Although *Rhizoctonia solani* was one of the less frequently isolated fungi, pathogenic isolates of this fungus usually reduced seedling emergence to zero in both steamed and non-steamed soil.

Fungi isolated from the diseased roots of older sweetclover plants were also tested for pathogenicity on seedlings. As with the isolates from diseased seedlings, the pathogenic isolates from roots in descending order of their occurrence included *Fusarium spp.*, *Pythium ultimum*, *P. debaryanum*, and *Rhizoctonia solani*. Additional pathogenic isolates from roots included *Aschochyta imperfecta* Pk. and *Mycosphaerella lethalis* Stone, both of which induced a high percentage of pre-emergence blight and some post-emergence blight in steamed and non-steamed soil. The fungus *Phytophthora cactorum* was found to cause little pre-emergence but very considerable post-emergence seedling blight in steamed soil. On the other hand, little of either pre-emergence or post-emergence blight by this fungus could be demonstrated in non-steamed soil.

The Influence of Organic Material on Seedling Blight in Field Soils

In a test of its pathogenicity on sweetclover seedlings, an isolate of *P. cactorum* was cultured on rolled oats for 2 weeks, after which the mycelial mat was washed as free as possible from the medium and minced. This mycelium was mixed with steamed and with non-steamed soil in the proportion of one culture to two 5-inch pots of soil. Similar quantities of inoculum that had been killed by steaming were added to other pots of soil. The checks consisted of untreated steamed and non-steamed soil. Sweetclover was sown in duplicate pots of each soil and treatment at the rate of 50 seeds per pot. The emergence and the stand 2 weeks after emergence are shown in Table 5. In this test the seedling emergence in the steamed soil was uniformly high regardless of treatment, but within 2 weeks post-emergence blight severely reduced the stand in the soil to which viable *P. cactorum* had been added. Although emergence in the untreated, non-steamed check soil was about equal to that in steamed soil, few seedlings emerged in the non-steamed soil to which either the viable or steamed mycelial mat had been added. The fact that the steamed mycelial

TABLE 5.—THE EFFECT OF VIABLE AND NON-VIABLE INOCULUM OF *P. cactorum* ON SEEDLING STAND IN STEAMED AND NON-STEAMED SOIL

	Percentage seedling stand			
	Steamed soil		Non-steamed soil	
	Emergence	Two weeks later	Emergence	Two weeks later
Viable *P. cactorum* mycelial mat	62	16	1	0
Steamed *P. cactorum* mycelial mat	66	64	4	4
Check	67	67	65	60

mat had a strong effect in non-steamed soil, but little effect on emergence in steamed soil, indicated that the mycelial mat must have favoured the pathogenic fungi in the non-steamed soil.

Another test was conducted to determine the relative effects of various media and variously treated cultures of *P. cactorum* on the incidence of seedling blights in steamed and non-steamed soils. The cultures and media used, and the resultant stands are all listed in Table 6. The results show that seedling emergence was between 56 and 72 per cent in all treatments in steamed soil, but post-emergence blight caused a drastic reduction of stand in all treatments that included viable mycelium of *P. cactorum*. In the non-steamed soil, on the other hand, only 3 treatments permitted an emergence as high as 56 per cent to 62 per cent. These included the treatments with potato dextrose agar alone, a culture of *P. cactorum* on potato dextrose agar, and the check. Next to the highest seedling stands

TABLE 6.—THE EFFECT OF VARIOUS CULTURES OF *P. cactorum* AND VARIOUS PLANT MATERIALS ON THE SEEDLING EMERGENCE AND THE POST-EMERGENCE BLIGHT IN STERILIZED AND NON-STERILIZED SOILS

	Percentage seedling stand			
	Steamed soil		Non-steamed soil	
	Emergence	Two weeks later	Emergence	Two weeks later
1. Check	72	72	57	57
2. Viable culture of *P. cactorum* on rolled oats	58	17	3	2
3. Steamed culture of *P. cactorum* on rolled oats	66	64	0	0
4. Viable culture of *P. cactorum* on rolled oats (excess medium washed away)	64	12	14	12
5. Steamed culture of *P. cactorum* on rolled oats (excess medium washed away)	72	72	8	8
6. Culture of *P. cactorum* on p.d.a.	67	15	62	46
7. Potato dextrose agar	72	72	56	49
8. Rolled out medium	68	52	0	0
9. Steamed cornmeal	62	53	11	10
10. Crushed wheat	56	51	0	0
11. Sweetclover roots (steamed)	72	72	21	21
12. Sweetclover roots (non-steamed)	61	61	39	26

occurred in the soils to which had been added steamed sweetclover roots (21 per cent emergence), and non-steamed sweetclover roots (39 per cent emergence). All of the other treatments, which included viable and non-viable (steamed) cultures of *P. cactorum* on rolled oats, viable and steamed mycelial mat preparations of *P. cactorum*, rolled oats, cornmeal, and crushed wheat, caused the seedling emergence in the non-steamed soil to be reduced to between 0 and 14 per cent. These results substantiate those of the preceding experiment in that relatively large quantities of organic materials encouraged severe seedling blight in non-steamed field soil.

An additional test was made with the purpose of comparing the effect of organic media on seedling emergence in 3 types of soil. Rolled oats, cornmeal, and finely chopped sweetclover roots were added to pots of non-steamed sand, clay, and sandy-loam, and to steamed sandy-loam. One hundred sweetclover seeds were sown in each of two 5-inch pots of each soil treated with the various amendments (15 gm. per pot) and also in untreated checks. The results are summarized in Table 7. Emergence was between 59 per cent and 67 per cent in the untreated clay and non-steamed and steamed sandy-loam, but was as low as 29 per cent in the sandy soil. None of the treatments, except possibly the sweetclover roots, had any appreciable effect on seedling emergence in the steamed sandy-loam. All treatments markedly reduced emergence in the sandy soil, and although all of them reduced emergence in the non-steamed clay and sandy-loam, the percentage of reduction was highest for rolled oats and least in the case of the sweetclover roots. These results indicate that the effect on seedling emergence of the addition of organic materials to the soil environment depends not only on the type of material but also on the soil.

TABLE 7.—THE EFFECT OF ORGANIC MATERIALS ON THE EMERGENCE OF SWEETCLOVER SEEDLINGS IN DIFFERENT SOILS

Amendment	Percentage seedling emergence*			
	Field sand	Clay	Non-steamed sandy-loam	Steamed sandy-loam
Untreated check	29.0	64.0	59.0	67
Rolled oats	2.5	1.5	11.0	60
Cornmeal	2.0	20.5	38.0	72
Sweetclover roots	4.0	24.0	46.5	51

* Percentage emergence from 200 seeds in each treatment.

A limited number of isolations were made from soils in which pre-emergence blight had been increased by the addition of rolled oats and other plant materials. *Pythium spp.* and *Fusarium spp.* were the most common isolates. Further study would be necessary to determine the relation of these and other organisms to seedling blight in field soils in which various plant materials have been incorporated.

Crop Sequence in Relation to Seedling Blight

In Essex county sweetclover frequently precedes corn in the rotation. Consequently a number of fields have been found in which sweetclover has been sown every 4 years, some every 3 years, and several as often as every

2 years. Unfortunately, however, in many of these cases the stands of sweetclover have tended to become less satisfactory, with considerable seedling failure consistently occurring in them. Seedling failure has also occurred when sweetclover has been sown in fields immediately after an alfalfa crop. In one instance an extremely sparse stand of sweetclover was obtained in the spring on that portion of a field where a crop of alfalfa had been ploughed under the preceding fall, but a good stand was obtained on the remainder of the field where no alfalfa had grown. These facts indicate that the crops preceding sweetclover in the rotation may strongly influence the severity of seedling blight in sweetclover.

The influence of various preceding crops on the severity of seedling blight in the sweetclover crop was tested in a greenhouse experiment. Clay soils from 2 sources were employed in the experiment. Soil A was from a field in which sweetclover had been grown from time to time, and Phytophthora rootrot was quite severe in the current year crop of sweetclover. Soil B was from a wheat field that had not grown a crop of sweetclover for many years. Seed of soybean, wheat, and corn, washed roots of 4-months-old sweetclover and alfalfa plants that had been grown in steamed soil, and washed, disease-free roots of red clover plants that had grown in the field were planted in 10-inch pots of each soil. The pots were kept in the greenhouse under favourable growing conditions for 2 months. Then in mid-October they were placed outdoors where they were exposed to mild fall and winter weather until the end of December. They were then moved into the greenhouse where the sweetclover, alfalfa, red clover, and wheat resumed growth. Ten weeks later the plants, except for part of the top growth of the sweetclover, alfalfa, and red clover, were cut into small pieces and incorporated with the soil in which they had grown. Two weeks later sweetclover seed was sown in all pots of soil. The seedling emergence, post-emergence blight, and the stand of seedlings 2 weeks after emergence were recorded, and the results were analysed statistically (Table 8). As an indication of the emergence to be expected in disease-free soil, 400 sweetclover seeds were also sown in samples of steamed soil from series A. The emergence from the 400 seeds is also given in Table 8.

The average seedling stand was somewhat higher in the soil from the wheat field than in that from the sweetclover field. In both soils there were highly significant differences between the stands obtained after different crops. The seedling stands after wheat and corn were slightly lower than in steamed soil. In soil A the stand after soybeans was significantly lower than after wheat and corn. The lowest seedling stands in both soils were obtained after sweetclover, alfalfa, and red clover, where they were even significantly lower than after soybeans. Only a small percentage of post-emergence blight occurred in any of the pots, but considerable pre-emergence blight was detected when the soil was searched for sprouted seeds, indicating that pre-emergence blight was the main cause of the differences in emergence after the various crops.

Pathogenic fungi were isolated in the following manner from seedlings that became blighted before emergence. Sweetclover seeds were sown in Petri dishes and covered with soil from the sweetclover, alfalfa, and red clover series. The germination of the seed could be observed conveniently through the bottoms of the Petri dishes. As soon as the seedling roots

TABLE 8.—EMERGENCE AND STAND OF SWEETCLOVER SEEDLINGS IN TWO FIELD SOILS FOLLOWING VARIOUS CROPS

Preceding crop	Soil A			Soil B		
	From a sweetclover field			From a wheat field		
	Emergence[1]	Post-emergence blight	Seedling stand	Emergence	Post-emergence blight	Seedling stand
Sweetclover	15.0	1.8	13.2[2]	23.6	0	23.6[3]
Alfalfa	28.0	2.5	25.5	35.8	1	34.8
Red clover	21.4	1.0	20.4	22.0	0	22.0
Soybeans	43.5	0.0	43.5	59.7	0	59.7
Wheat	59.7	1.0	58.7	67.1	0	67.1
Corn	60.4	0.7	59.7	66.9	0	66.9
Steamed[4]	72.8	0.0	72.8			

[1] Percentage emergence, blight, and stand in 4 replicates with 200 sweetclover seeds sown in each.
[2] Least significant difference at 5 per cent point is 14.2, at 1 per cent point is 19.7.
[3] Least significant difference at 5 per cent point is 23.1, at 1 per cent point is 32.0.
[4] Percentage emergence from 400 seeds.

began to turn brown, thus indicating parasitic attack, they were removed from the Petri dish, washed free of soil, and either surface-sterilized with 1 : 1000 solution of mercuric chloride or washed in several changes of sterile water, and then planted on agar media. Cultures of fungi that grew from diseased roots were tested for pathogenicity. The predominating pathogen obtained in this way was a highly virulent form of *Rhizoctonia solani*, which was evidently the main cause of pre-emergence blight in these soils. Smaller proportions of *Fusarium spp* and *Pythium spp.* were also obtained.

It is concluded from the above field observations and greenhouse experiments that the preceding crops may have a highly significant effect on the subsequent stand of sweetclover seedlings on some soils because of their influence on the incidence of seedling blight.

DISCUSSION

Prior to the more intensive investigations of sweetclover failure in southwestern Ontario here reported, it seemed most difficult to reconcile the contradictory but nonetheless emphatic statements of reliable growers regarding the nature and time of occurrence of the trouble with the usual activity of a single disease. While, on the one hand, a considerable number declared that sweetclover failure on their farms occurred about the time of, or soon after, emergence of the seedlings, numerous others maintained that the trouble did not become apparent until the spring of the second season, when large populations of plants, usually in patches, completely disappeared from fields in which stands had previously been uniform and satisfactory.

The present investigations have vindicated both statements, the explanation resting in the widespread occurrence of two distinct troubles, one of which represents the seedling phase of the so-called "failure", and the other the more spectacular rootrot phase of mature plants. The present investigations of the rootrot caused by *P. cactorum* have further explained the phenomenon of the rapid rotting and disappearance of plants during the early spring by the finding that a relatively low optimum temperature

favours the activity and growth of the causal organism. Though the investigation of the effect of other factors on the rootrot, such as type of soil and moisture content, failed to reveal clear-cut relationships, nevertheless, it is felt that further experimentation would likely yield information of value.

The fact that several fungi appeared to be consistently involved in the seedling phase of the disease is not surprising when viewed in the light of similar diseases of other hosts. Although the fungus, *P. cactorum*, is responsible for the rootrot of mature plants and is capable of attacking seedlings in sterilized soil, it has not been found associated with the seedling phase of the "failure". This phenomenon suggests an undetermined mechanism protecting the host from this fungus during the earliest stages of growth in field soils. The results of the preliminary observations and tests concerning the effect of organic matter and the preceding crops on the incidence of seedling blights strongly suggest that crop rotation may have a quite important bearing on sweetclover failure in southwestern Ontario, and as such should be investigated more comprehensively.

SUMMARY

During recent years sweetclover has become an uncertain crop in southwestern Ontario. In some cases, unsatisfactory stands were the result of calcium deficiency, sweetclover weevil infestation, or adverse weather conditions, but most of the failures observed in Essex and Kent counties in 1947-1949 were caused either by a rootrot disease or a seedling blight.

Phytophthora cactorum (Leb. and Cohn.) Shroet was the principal pathogen causing a rootrot which destroyed a high percentage of the plants in many fields each year. This rootrot proved to be the most destructive during late April, and soil temperature appeared to be the main factor influencing its seasonal severity. The soil temperature during late April, 1949, at Harrow ranged for the most part, from 6° to 16° C. Greenhouse experiments showed that severe infection resulted from artificial inoculation when soil temperatures were 20° C. or lower. It was also found that the fungus grew most rapidly in culture at temperatures between 12° and 20° C., with the optimum temperature at approximately 16° C. Hence, it appears that relatively low soil temperatures, such as those occurring during late April and early May in Essex county, strongly favour the vegetative growth and pathogenicity of *P. cactorum* attacking sweetclover. The texture of the soil also appears to influence the severity of the disease. Rootrot occurred more frequently and was more severe in heavy than in light-textured soils.

Seedling blight caused mild to severe reductions of sweetclover stands in many fields. Much of the blight was of the pre-emergence type. The fungi most frequently isolated from diseased seedlings included *Fusarium spp.*, *Pythium debaryanum* Hesse, *P. ultimum* Trow., and *Rhizoctonia solani* Kuehn. The addition of various starchy plant materials or dead sweetclover roots to field soil caused significant increases in pre-emergence blight of sweetclover seedlings. The crops preceding sweetclover appeared to have an important relation to pre-emergence seedling blight, because it was more severe after sweetclover, alfalfa, or red clover than after soybeans, corn, or wheat.

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DILUTION PROCEDURES FOR PLATE COUNTS ON DRY MILKS[1]

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Two important factors in the dilution procedures for determining plate counts on dry milks are: (a) the kind of diluent used for reconstituting the dry milks, and (b) the temperature of the diluent at the time of adding the test portion of the dry milk. Sterile water is most commonly used for reconstituting dry milks for bacteriological analysis, but other diluents have been recommended and used. The 9th edition of Standard Methods for the Examination of Dairy Products (1) allows the optional use of 0.1 N lithium hydroxide as a reconstituting medium, and Prickett and Miller (12) and Downs *et al.* (4) have recommended 0.1N lithium hydroxide as a diluent for dry milks which are difficult to put into solution in water. Hiscox (7) and Higginbottom (6) used one-quarter strength Ringer's solution for reconstituting dry milks for bacteriological analysis, while Garrison (5) has recommended the use of a 1.25 per cent sodium citrate solution.

While it has been regarded by some (1, 12) that 0.1N lithium hydroxide is non-toxic to bacteria in dry milk, Garrison (5), Cone and Ashworth (3) and White (16) have found this concentration of lithium hydroxide to be definitely germicidal to bacteria in the usual types of dry milks, and the plate counts, especially of spray-dried milks, were reduced considerably.

The temperature of the diluent is also a factor affecting the plate counts on dried milks. Several investigators (6, 7, 16) have found that higher counts were obtained on dry milks when the diluent was at 50° C. rather than at room temperature (20° to 25° C.). Cone and Ashworth (3) showed that reconstituting the dry milks at 45°, 50°, and 55° C. rather than at room temperature (22° to 25° C.) resulted in higher counts, while Speck and Myers (14) showed that reconstituting spray-dried milk cultures of *Lactobacillus bulgaricus* at 37°–50° C. activated cells that failed to grow when the diluent was at 21° to 25° C. The 9th edition of Standard Methods for the Examination of Dairy Products (1) allows for the warming of dilution blanks to 35° C., while the Methods of Analysis of the American Dry Milk Institute (2) states that it may be helpful to temper the dilution blanks at not over 40° C. to obtain a satisfactory homogeneous dispersion of the sample but that the test portion should not be in contact with the water at a temperature above 37° C. for more than 15 minutes.

This study was undertaken to obtain additional information on the effect of 0.1N lithium hydroxide on the plate counts of dry milks when reconstituted at 50° C.; and also on the viability of pure cultures of various organisms isolated from dry milks and other sources.

METHODS AND MATERIALS

The samples of dry milks examined in this study were commercially dried milks sent to this laboratory for routine analysis in connection with grading or for check testing.

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The dry milks were reconstituted by weighing 13 grams of whole milk powder or 10 grams of skim-milk powder directly into duplicate dilution bottles of 100 ml. of sterile water and 0.1N lithium hydroxide which had been tempered previously in a water bath at 50° C. After reconstitution of the dry milks by thoroughly shaking 50 times, one set of the reconstituted milks was placed in the water bath at 50° C. and held for 15 minutes before plating, while the duplicate set was held at room temperature for the same time. Further dilutions of the milks reconstituted in 0.1N lithium hydroxide were made in sterile distilled water. Duplicate plates of at least two dilutions were prepared.

TABLE 1.—COMPARATIVE COUNTS OF DRY MILKS RECONSTITUTED IN WATER AND IN 0.1N LITHIUM HYDROXIDE

Sample No.	Reconstituted in Water		Reconstituted in 0.1N LiOH		Per cent Decrease	
	A	B	A	B	A	B
Roller-Dried Skim-milk						
JFC 23	13,000	28,000	4,600	6,600	64.7	76.4
JFC 26	510	660	250	190	51.0	71.2
CRK 26	900	1,400	450	550	50.0	60.7
HD 43	750	730	380	430	49.4	41.0
HD 44	3,600	6,500	1,400	1,300	61.0	80.0
HD 46	2,000	1,900	490	850	75.5	55.3
HD 47	1,500	1,600	600	530	60.0	67.0
HD 48	1,700	1,700	590	780	65.3	54.0
HD 49	1,900	1,900	970	950	49.0	50.0
EL 390	2,700	1,300	600	650	78.0	50.0
Spray-Dried Skim-milk						
EL 140	19,000	19,000	8,800	10,000	53.7	47.4
EL 141	280,000	310,000	13,000	28,000	95.4	91.0
DRC 120	4,900	4,400	1,800	1,900	63.3	56.8
DRC 133	140,000	180,000	35,000	49,000	65.0	72.8
DG 45	22,000	24,000	1,200	1,200	94.6	95.0
WAB 314	210	220	65	35	69.0	86.0
LK 52	85,000	120,000	3,000	1,500	97.0	98.7
LK 57	29,000	26,000	200	500	99.0	98.1
JRB 14	76,000	84,000	2,300	5,500	97.0	93.5
JRB 15	7,800	12,000	430	680	94.5	94.3
DRC 279	34,000	38,000	6,000	13,000	82.4	65.8
DRC 280	38,000	39,000	9,100	9,000	76.0	77.0
Spray-Dried Whole Milk						
AS 1	28,000	32,000	7,800	12,000	72.0	62.5
AS 7	130,000	140,000	36,000	37,000	72.4	73.6
2313	24,000	27,000	2,200	2,000	90.9	92.6
2315	71,000	74,000	700	900	99.0	98.8
2314	14,000	12,000	150	160	99.0	98.7
2185	58,000	62,000	1,100	4,000	98.0	93.7
2163	6,800	7,000	110	340	98.4	95.1
2164	15,000	14,000	4,300	6,400	71.4	54.3
2165	11,000	10,000	1,100	1,300	90.0	87.0
2188	13,000	15,000	3,300	3,000	74.7	80.0
2768	1,900	1,900	120	80	93.7	95.7
2771	13,000	13,000	1,100	2,000	91.0	84.6

All samples reconstituted in diluent at 50° C.

A Series: Held in water bath at 50° C. for 15 minutes before plating.
B Series: Held at room temperature, 22°–26° C., for 15 minutes before plating.

The medium was "Difco" tryptone glucose extract agar plus 1 per cent fresh skim-milk and the plates were incubated at 32° C. for 48 hours. Counts were made with a Quebec Colony Counter and reported as the standard plate count per ml. on a reconstituted basis.

RESULTS

The Effect of 0.1N Lithium Hydroxide on the Plate Counts of Dry Milks

The data in Table 1 show that there was a marked reduction in the plate counts of individual samples of dry milks reconstituted in 0.1N lithium hydroxide as compared to water. The percentage reduction ranged from 41 to 80 per cent for roller-dried milks and from 47 to 99 per cent for the spray-dried skim and whole milk powders. A summary of the average plate counts for the different types of powders is given in Table 2. The average reduction for roller-dried powders was slightly more than 60 per cent, and was over 80 per cent for both skim and whole milk spray-dried powders.

TABLE 2.—SUMMARY OF THE AVERAGE PLATE COUNTS OF DRY MILKS WITH DIFFERENT DILUENTS

Type of Dry Milk	No. of Samples	Reconstituted in Water		Reconstituted in 0.1N LiOH		Av. % Decrease	
		A	B	A	B	A	B
Roller Skim-milk	10	2,900	4,600	1,030	1,300	60.3	60.5
Spray Skim-milk	12	61,000	71,000	6,700	10,000	82.2	81.4
Spray Whole Milk	12	32,000	34,000	4,500	5,800	87.5	84.7

All samples reconstituted in diluent at 50° C.
A Series: Held at 50° C. for 15 minutes before plating.
B Series: Held at room temperature for 15 minutes before plating.

The Effect of 0.1N Lithium Hydroxide on Pure Cultures

To determine the effect of 0.1N lithium hydroxide on pure cultures, a number of typical colonies were picked from plates of various milks. Many of the cultures were Gram positive cocci from the spray-dried milks, while a number of Gram positive spore-forming rods were isolated from plates of roller-dried milks. In addition, a number of pure cultures of *Micrococcus* species from the Division's collection were tested. Starters of lactic streptococci and a few cultures of Gram negative coliforms were included in the trials.

The organisms were cultured on T.G.E. agar slants for 24 to 48 hours and some of the growth was suspended in sterile water or sterile skim-milk. In the case of the starters, about 0.1 ml. of an active coagulated starter was mixed with 10 ml. of sterile skim-milk. After thorough shaking, 0.1 ml. of the original suspension in water or milk was transferred to 99 ml. blanks of water and 0.1N lithium hydroxide at 50° C., and allowed to stand at room temperature for 10 to 15 minutes before plating. Further dilutions were made in water blanks at room temperature. Duplicate plates were poured to give 1 : 10,000, 1 : 100,000 and 1 : 1,000,000 dilutions of the original suspension of the organisms.

TABLE 3.—THE EFFECT OF 0.1N LITHIUM HYDROXIDE ON VARIOUS ORGANISMS IN PURE CULTURE

Culture	Plate Count of Original Suspension		Per cent decrease
	In Water	In 0.1N LiOH	
LK 52-1, Gram + coccus	9,500,000	900,000	90.5
LK 52-4, Gram + coccus	1,200,000	10,000	99.1
LK 57-7, Gram + coccus	930,000	20,000	97.9
2313-1, Gram + coccus	30,000,000	10,000,000	66.6
2313-3, Gram + coccus	132,000,000	40,000	99.9
2315-2, Gram + coccus	10,000,000	2,600,000	74.0
AKS 1, Gram + sporing rod	35,000,000	6,000,000	83.0
AKS 6, Gram + sporing rod	5,000,000	150,000	97.0
M. roseus	1,000,000	nil	100.0
M. sp. from ropy milk	980,000	nil	100.0
M. pyogenes var. aureus	310,000,000	16,000,000	95.0
Starter culture M.	650,000	2,000	99.7
Starter culture EDM.	420,000	nil	100.0
Strep. faecalis	143,000,000	1,200,000	99.2
Coliform	618,000,000	10,000	>99.9
Coliform	680,000,000	20,000	>99.9

Comparative plate counts from water and 0.1N lithium hydroxide blanks of some of the cultures are given in Table 3. With cultures of cocci, contact with 0.1N lithium hydroxide destroyed from 66 to 100 per cent of the cells, as shown by the plate counts. Lithium hydroxide also showed germicidal activity against the spore-forming Gram positive rods, although when the cultures were in the spore stage mainly, there was a slight increase in the plate counts from the lithium hydroxide blanks. It is probable that the spores of these organisms are more resistant to lithium hydroxide than the vegetative cells. With the lactic streptococcus organisms in starters and Gram negative coliform cultures, the lithium hydroxide destroyed over 99 per cent of the cells, as indicated by the plate counts of the original suspensions.

The pH of Reconstituted Milks

The pH values of reconstituted milks in both water and 0.1N lithium hydroxide were determined on the Beckman pH meter and results are shown in Table 4. The average pH for the undiluted milks reconstituted in water was 6.58 as compared to 10.6 for milks reconstituted in 0.1N lithium hydroxide. When dilutions of 1 : 10 and 1 : 100 were made in sterile water from the lithium hydroxide blanks, the average pH values were 10.55 and 9.42, respectively. These results are almost identical to those obtained in a previous study (16).

A few pH determinations were made on the Beckman pH meter of reconstituted milk-agar mixtures by adding 1 ml. of the 1 : 10 dilution of the milks to 8 ml. of the medium. The pH of the inoculum-agar mixture from water was about 6.7, while the pH of the inoculum-agar mixture from lithium hydroxide was approximately 6.95. The pH of the medium alone was 6.75.

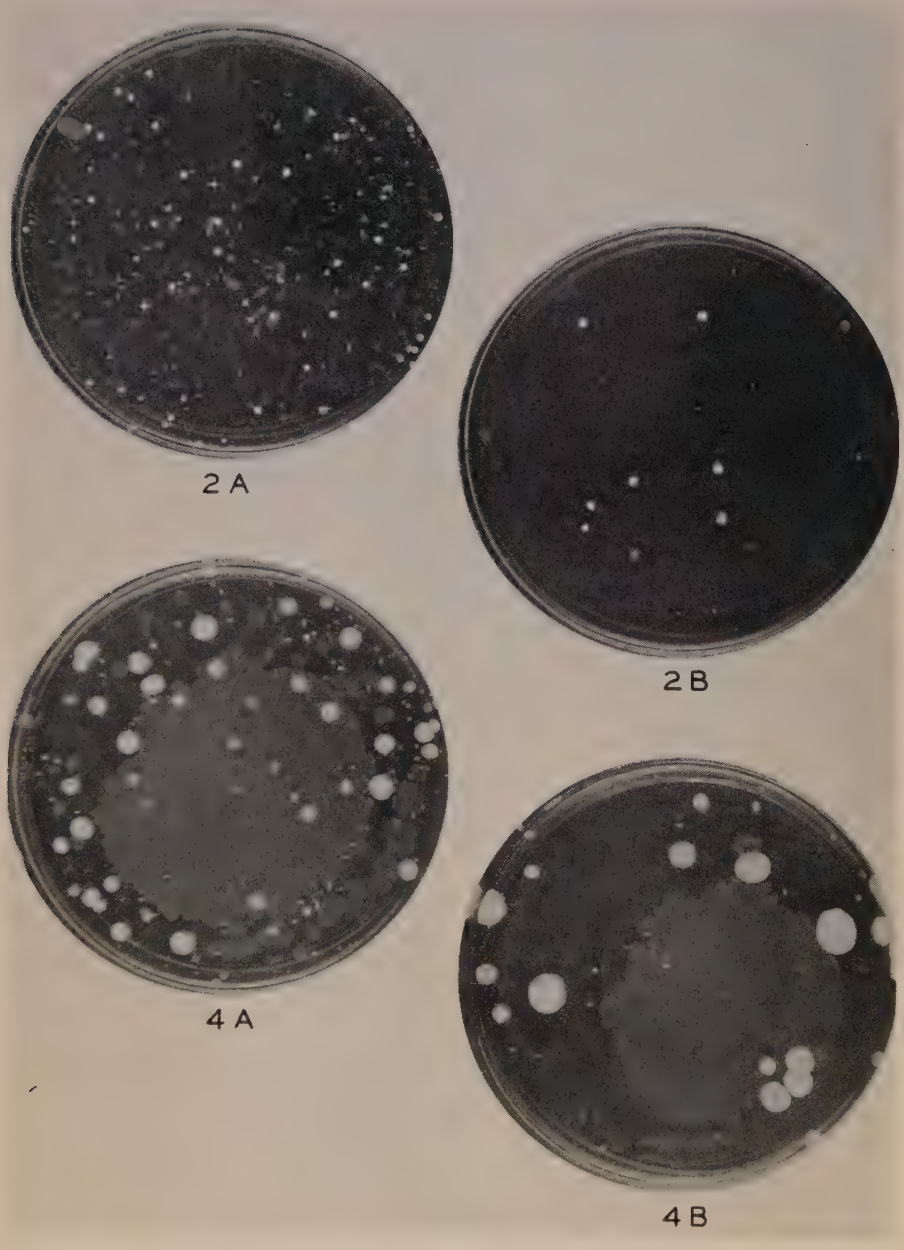

FIGURE 1. The effect of 0.1N LiOH on the plate count of pure cultures. 2. *M. candidus*. 4. Gram positive spore-forming bacillus. *A* samples reconstituted in water. *B* samples reconstituted in 0.1 N LiOH.

TABLE 4.—pH VALUES OF RECONSTITUTED DRY MILKS

Sample	Type of Milk	Reconstituted in Water			Reconstituted in 0.1N LiOH		
		Not diluted	1 : 10	1 : 100	Not diluted	1 : 10	1 : 100
2186	Whole spray	6.60		7.25	10.70	—	9.75
2163	Whole spray	6.55	6.80	—	10.72	10.60	—
2188	Whole spray	6.55	—	6.90	10.50	—	9.55
2768	Whole spray	6.60	7.10	—	10.62	10.60	—
JRB 14	Skim spray	6.60	—	7.00	10.50	—	9.1
JRB 15	Skim spray	6.59	7.00	—	10.60	10.60	—
DRC 279	Skim spray	6.55	—	7.13	10.50	—	9.6
DRC 280	Skim spray	6.52	—	6.91	10.55	10.45	—
RL 104	Skim roller	6.70	7.20	—	10.67	10.67	—
EL 390	Skim roller	6.55	7.02	—	10.70	10.40	—
Average		6.58	7.0	7.03	10.6	10.55	9.42

The Temperature of Reconstituted Milks at Plating

Temperature readings of a number of milks reconstituted at 50° C., taken just after the test portion was removed for plating, showed that when the reconstituted milks were held at 50° C. the average temperature was about 45° C., while the duplicate samples held at room temperature averaged about 37° C. The charge of 10 or 13 grams of dry milk reduced the temperature of the reconstituted milks to approximately 34° to 39° C., depending on the air temperature at the time. When the reconstituted milks are held at room temperature before plating, the test portion of the dry milk will never be in contact with the reconstituting medium at a temperature over 40° C. for more than a few seconds.

Although the average counts are slightly higher when the milks are held at room temperature rather than at 50° C. before plating, as shown in Table 2, the difference is not considered to be significant.

The Dispersion of Dry Milks in Water and in Lithium Hydroxide

As in previous studies, no difficulty was experienced in obtaining good dispersion of the dry milks, even roller-dried powders, in water at 50° C. In most cases, there appeared to be better dispersion in water than in lithium hydroxide, as the powder seemed to form small balls in lithium hydroxide which were never completely dispersed by the time of plating This condition was never apparent with water. Although there were some undissolved powder particles in the 1 : 10 dilution plates of roller-dried milks reconstituted in water, no particular difficulty was experienced in counting the plates when the usual types of bacterial colonies were present. If pin-point colonies are present, it is advisable to examine the plates with the low power lens of a microscope or with a stereoscopic microscope giving a magnification of 6 or 12.

DISCUSSION

Bacterial counts on the types of milk powder used in this study were significantly reduced when 0.1N lithium hydroxide was used as the reconstituting medium. Lithium hydroxide was also germicidal to pure cultures of micrococci and spore-forming Gram positive rods isolated from milk powders, as well as to lactic streptococci in starters and to coliform organisms. This is an important consideration from a grading standpoint as the use of 0.1N lithium hydroxide would lower the count of many samples sufficiently to put the powder into first grade by Canadian standards, whereas the plate count of the powder, if reconstituted in water, would place the sample below second grade. This is especially true for spray-dried powders. Although the average percentage reduction in the plate counts of roller-dried powders was approximately of the same order as for spray powders, the use of lithium hydroxide has less significance from a grading standpoint due to the general low level of plate counts of roller-dried powders.

Where bacteriological plate counts of dry milks are used as an index of plant and equipment sanitation, the results obtained from lithium hydroxide solutions of the powder would be misleading in many cases and give a false impression of the sanitary conditions of equipment and plant.

The germicidal effect of lithium hydroxide is due undoubtedly to the high alkalinity (high concentration of hydroxyl ions) in the milk-lithium-hydroxide mixture. Several investigators (8, 9, 10, 11, 13, 15), using pure cultures of test organisms or mixed cultures of organisms associated with milk utensils and containers, found that alkaline solutions in the range of pH 10.0 to 12.0, and even as low as 9.0, destroy a high proportion of the viable organisms in 1 to 10 minutes. The studies also indicated that as the alkalinity or pH increased, the time necessary to destroy the organisms decreased.

As the pH of the milk-lithium hydroxide mixture is about 10.6 and further dilutions of 1 : 10 and 1 : 100 in water have pH values of about 10.5 and 9.4, respectively, the high concentration of hydroxyl ions is considered to be the main factor in the germicidal effect of the lithium hydroxide solution on the bacteria of milk powders. However, when the portion of a milk-lithium hydroxide dilution was mixed with 8 ml. of melted agar, the buffering capacity of the latter reduced the pH to below 7.0, where it would no longer have a deleterious effect.

There is apparently some apprehension as to the effect of tempering the reconstituting medium to 50° C. on the plate counts of dry milks, as the latest methods of the A.P.H.A. and the American Dry Milk Institute recommend heating the dilutions to 35° C. and not over 40° C., respectively. However, heating the diluent to 50° C. appears to activate the viable bacteria so that they grow rather than being inhibited, and plate counts are higher. It has therefore been routine procedure in the author's laboratory to preheat the diluent to 50° C. for determining plate counts of dry milk.

If the bacteriological analysis of dry milks is to attain full usefulness in the grading and sanitary control of production of dry milks, the technique should give the highest estimate of the viable organisms in the product. From this viewpoint, these studies indicate that it is preferable to reconstitute dry milks in sterile water previously tempered to 50° C. and hold

at room temperature until plated. Lithium hydroxide is not recommended as a reconstituting medium for the bacteriological examination of the usual types of dry milks because of its germicidal effect.

SUMMARY

Plate counts are greatly reduced when 0.1N lithium hydroxide is used as a diluent for reconstituting dry milks. A large proportion of the cells of pure cultures of various bacteria are also destroyed when placed in 0.1N lithium hydroxide.

The pH of the reconstituted milks in 0.1N lithium hydroxide was approximately 10.6 as compared to 6.6 for water reconstituted milks. The germicidal action of 0.1N lithium hydroxide is considered, therefore, to be due to the high hydroxyl ion concentration of the milk-lithium hydroxide mixture.

When test portions of dry milk are added to the diluent at 50° C., the temperature of the reconstituted milk is lowered to below 40° C. and, if held at room temperature, is approximately 35° to 37° C. at the time of plating.

Dilution procedures recommended for the bacteriological examination of dry milks are as follows: Reconstitute the dry milk in sterile water blanks previously tempered to 50° C. and hold the reconstituted milk at room temperature until plated; further dilutions to be made in sterile water at room temperature.

The use of 0.1N lithium hydroxide is not recommended for reconstituting the usual types of dry milks for determining plate counts.

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INHERITANCE IN WHEAT OF STEM RUST RESISTANCE DERIVED FROM *AGROPYRON ELONGATUM*[1]

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INTRODUCTION

In recent years it has become increasingly apparent that stem rust resistance in wheat from one source will not solve the rust problem permanently. With the advent of new physiologic races or biotypes of existing races, the plant breeder may find it necessary to seek new sources of resistance. Some of the available sources of resistance other than the varieties H-44-24 and Hope which may prove of particular interest and value to Western wheat breeding programs are listed by Elliott (4) and McFadden (10).

Successful crosses have been made between *Triticum vulgare* and *Agropyron elongatum*. A number of lines developed from the intergeneric cross backcrossed to *T. vulgare* have shown a high degree of stem rust to a mixture of races under artificial epidemic conditions at the University of Saskatchewan. Since the bread wheat variety, Chinese, used in the development of the resistant lines is susceptible under the same epidemic conditions, the source of resistance is considered as being derived from the *Agropyron*.

This paper deals with the mode of inheritance of the new source of resistance.

REVIEW OF LITERATURE

Four decades ago Biffen (1, 2) first demonstrated that the resistance of wheat to a particular disease (*Puccinia glumarum*) was inherited according to Mendelian laws. Since then an enormous amount of information concerning the inheritance of disease resistance in plants has been accumulated.

The genetical background of a number of new varieties of spring wheat resistant to stem rust in the Canadian West has been presented by Neatby (11). In his paper he points out that three varieties in commercial production in Canada (Renown, Regent and Apex) possess the H-44-24 type of stem rust resistance. Apex possesses in addition to the H-44-24 type of resistance, additional genes for resistance from Double Cross.

The rust resistance of H-44-24 has been shown to be a "mature-plant resistance" (5). The mature-plant resistance of H-44-24 is dominant and governed by a single pair of genes (5, 6, 12).

The rust resistance of Apex, although coming from two sources (11) may be considered as being the mature-plant type of resistance as long as race 56 of stem rust is one of the predominant races in Western Canada. The predominance of race 56 has been indicated by Johnson *et al.* (9) and Newton (13). The seedling susceptibility of Apex to race 56 has been demonstrated by Newton *et al.* (14).

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The stem rust resistance of Thatcher differs from that of H-44-24. Neatby (11) indicated that Thatcher's seedling type resistance is governed by more than a single gene and that its field resistance is recessive and determined by two or more genes. Hayes *et al.* (7) and Neatby and Goulden (12) indicated that three or more genes for resistance are present in Thatcher. Recently Swenson *et al.* (17) in a study of the cross Thatcher × Triunfo, concluded that at least two or three recessive genes for resistance were involved in differentiating between the resistant reaction of Thatcher and the susceptibility of Triunfo.

The variety Red Egyptian furnishes a different type of resistance to stem rust than does either H-44-24 or Thatcher. Johnson and Newton (8) found McMurachy, Kenya R. L. 1373, Red Egyptian, N. A. 95 and Iumillo to be either immune or resistant to races 15, 29 and 56 in both the seedling and mature-plant stages. Shebeski (15) in a study of diallel crosses between Marquis, Kenya, McMurachy and Red Egyptian, concluded that in the three varieties, Kenya, McMurachy and Red Egyptian, one recessive gene pair governed resistance to both 15B and to 41 common races including race 56.

At a meeting of the Associate Committee on Plant Breeding held at Winnipeg in February of 1950, Dr. Sears*, in discussing nullisomics, stated that chromosome XX of Red Egyptian has a factor for resistance to races 17 and 56, while chromosome VI has a factor for resistance to races 38, 56, and 139. It could be possible, as indicated in a report by Shebeski (15), that the effect of one of the pairs of genes was masked by the use of the mixture of 41 races or, in other words, one of the two genes reported by Sears is not as effective as the other.

MATERIALS AND METHODS

The varieties of wheat used in this study were Apex, Thatcher, Red Egyptian and Perennial wheat (P.W. 357-5). The wheat termed Perennial resulted from the cross Chinese × (Chinese × *A. elongatum*) made in 1939. It is a late maturing, long strawed, long spiked, awnless, stem rust resistant variety.

The following crosses were made in the greenhouse in the spring of 1949:

Apex × P.W. 357-5
Thatcher × P.W. 357-5
Red Egyptian × P.W. 357-5

The F_1 population of each cross was grown under artificial rust epidemic conditions in an irrigation nursery during the summer of 1949. In the fall of 1949 the F_2 population of each cross was sown in a greenhouse bed. The hybrid seeds were spaced one inch apart in the row and the rows were spaced two inches apart. All parental materials including Chinese wheat and also reserve F_1's were seeded in separate rows adjacent to each F_2 population. Each cross was separated by a row of Little Club wheat and a row of Montcalm barley. Over 4,000 seeds were sown in one bed which was 20 feet long and 4 feet wide. A similar method of planting was reported by Tapke (18) wherein 30,000 to 60,000 barley plants were grown for smut studies in a 420 square foot space in the greenhouse.

* Paper presented in February, 1950, at the 5th Annual Meeting of the Associate Committee on Plant Breeding.

The inoculum used was collected in 1949 from the inoculated irrigation nursery plots at Saskatoon. The races present were not identified. Johnson *et al.* (9) reported that from 100 wheat stem rust isolates studies in 1949, 69 per cent were race 56. Of the seven isolations made in Saskatchewan, all were determined to be race 56. Therefore it may be assumed that the uredospores collected for use in this study were mainly those of race 56.

The method of inoculation was a modification of the method described by Cherewick (3). When the seedlings were in the two-leaf stage, the bloom was removed by gently rubbing the moistened leaves between fingers. An uredospore suspension was prepared and was sprayed thoroughly over the seedlings. Finally a uredospore dust (one part uredospores to five parts talc) was dusted over the moistened leaves.

Before the inoculation was started the seed bed had been thoroughly watered. After the inoculation was completed, the bed was covered with a plastic tent. At the same time the air temperature was lowered from 70 degrees to 50 degrees F. The seedlings were incubated under the plastic tent for 48 hours.

Readings were taken two weeks after the date of inoculation. The epidemic results obtained from this inoculation method were very satisfactory. Fifty seedlings of Montcalm and fifty seedlings of Little Club, taken at random from the six rows scattered throughout the bed, were all susceptible indicating little or no escapes. The seedlings were classified according to scales suggested by Stakman *et al.* (16).

RESULTS

Apex and Chinese were susceptible in the seedling stage whereas Thatcher, Red Egyptian and P.W. 357-5 were resistant. The F_1 populations were resistant, indicating that at least one parent in each cross possessed factors dominant for resistance. There was a great range in the stem rust reaction of the F_2 progenies in the three crosses. A greater proportion of susceptible seedlings were obtained in the crosses Apex × P.W. 357-5 and Thatcher × P.W. 357-5 than in the cross Red Egyptian × P.W. 357-5.

The classification of rust reactions is given in Tables 1 and 2.

TABLE 1.—STEM RUST REACTION IN THE SEEDLING STAGE OF F_1'S AND PARENT VARIETIES

Variety or cross	Number of plants examined	Type of infection	Classification
Apex, Sask. 2177	40	4	Susceptible
Thatcher, Sask. 1720	40	0; – 1	Resistant
Red Egyptian, Sask. 2277	40	0;	Resistant
Chinese, Sask. 2050	10	4	Susceptible
P.W. 357–5	40	0; – 1	Resistant
Apex × P.W. 357-5	6	1 – 2	Resistant
Thatcher × P.W. 357-5	8	0;	Resistant
Red Egyptian × P.W. 357-5	10	0;	Resistant

TABLE 2.—STEM RUST REACTION OF F_2 SEEDLINGS

Cross	Type of Reaction						Classification*	
	0	0;	1	2	3	4	Resistant	Susceptible
Apex × P.W. 357-5	0	172	170	22	136	366	364	502
Thatcher × P.W. 357-5	0	164	256	28	194	359	448	553
Red Egyptian × P.W. 357-5	58	378	531	310	85	19	1077	104

* Types (0), (0;), (1) and (2) were grouped as resistant;
Types (3) and (4) were grouped as susceptible.

INTERPRETATION OF RESULTS AND DISCUSSION

Analysis of F_1 Results of the Apex × P.W. 357-5 Cross.

With Apex susceptible in the seedling stage and P.W. 357-5 resistant, and with the F_1 also classified as resistant, it may be assumed that resistance is dominant and came from P.W. 357-5.

The ratio of 364 resistant to 502 susceptible seedlings did not seem to fit satisfactorily any known ratio involving either one or two pairs of genes. Superficially the results seemed to fit a 7 : 9 ratio but since the F_1 was resistant, the two independent recessive gene hypothesis would not be used. Therefore, it was necessary to postulate either the presence of modifying factors affecting the action of one or two main genes or the presence of three or more sets of main genes affecting the reaction to rust. A 27 : 37 ratio was tested by chi-square and a very satisfactory fit was obtained. Chi-square was 0.010 which for $n=1$ has a P value between 0.90 and 0.95.

The results of this cross therefore indicate that the resistance of P.W. 357-5 is dominant and is governed by three complementary genes.

Analysis of F_2 Results of the Thatcher × P.W. 357-5 Cross

In this cross 1001 F_2 seedlings were examined. Resistant seedlings should have two sources of resistance, a 27 : 37 ratio of resistance versus susceptibility from P.W. 357-5 and a 1 : 63 ratio of resistance versus susceptibility from Thatcher (7, 11, 12, 17). This would mean 422 seedlings having P.W. resistance and 16 seedlings having Thatcher resistance. However, 7 of the 16 resistant Thatcher seedlings should also have the resistance of P.W. 357-5. Therefore, the theoretical ratio of resistance versus susceptibility should be 431 : 570. When this ratio was compared with the observed, a chi-square value of 1.18 was obtained which for $n=1$ lies between P values of 0.20 and 0.30. The satisfactory fit may be interpreted as supporting evidence for the hypothesis that three complementary genes with dominance are responsible for rust resistance in P.W. 357-5 and that three recessive genes are responsible for the resistance in Thatcher.

Analysis of F_2 Results of the Red Egyptian × P.W. 357-5 Cross.

In this cross 1077 F_2 seedlings out of 1181 were classified as resistant. The first hypothesis tested was that resistance was obtained from two sources; that is from the P.W. parent in a 27 : 37 ratio and from the Red

Egyptian parent in a 7 : 9 ratio. The theoretical ratio of 796 resistant to 385 susceptible thus obtained did not fit the observed results. Since a very high proportion of the seedlings were resistant, some of which were actually immune, it could be expected that there is complementary action for resistance between the P.W. 357-5 genes and the Red Egyptian genes. For the sake of convenience the P.W. genes were designated as P_1 , P_2 and P_3 , and the Red Egyptian genes were designated as r_1 and r_2 .

At least four different hypotheses of complementary action between the two types of resistance were tested before one was found that gave a theoretical ratio which satisfactorily fitted the observed results.

The theoretical ratio was obtained by postulating that the following gene combinations are responsible for resistance:

(a) P.W. 357-5 resistance which is $P_1P_2P_3$.
(b) Red Egyptian resistance which is r_1r_2 or r_2r_2 .
(c) Complementary action of r_1 with any one of P_1 , P_2 or P_3 .
(d) Complementary action of r_2 with P_1P_2 but not with P_1P_3 or P_2P_3.

In the above hypothesis it is assumed that r_1 is more potent than r_2 and that P_3 is less potent than either P_1 or P_2 . The theoretical ratio of resistant versus susceptible seedlings is 1067 : 114. The chi-square test for goodness of fit gave an χ^2 value of 1.06 which for $n = 1$ has a P value of approximately 0.30.

The method of working out the theoretical ratio is presented in Appendices A and B.

It is possible that some other type of complementary action may give a theoretical ratio which would also satisfactorily fit the observed results. However, the proposed hypothesis is particularly tenable since evidence has been presented that one of the Red Egyptian genes for resistance is more potent than the other.

In evaluating the F_2 results of the three crosses studied, and bearing in mind the information previously available on Apex, Thatcher and Red Egyptian, the hypothesis that P.W. 357-5 has three dominant complementary genes for resistance in the seedling stage to the stem rust races used is quite acceptable. The mode of inheritance of this new type of resistance is unquestionably different from the resistance of Apex, Thatcher, Red Egyptian and also H-44-24. The number of immune plants obtained in the F_2 of the Red Egyptian × P.W. cross suggests that combining the two sources of resistance can give more resistance than could be obtained from either parent alone. This may be of particular importance if new and more virulent races should develop.

SUMMARY

F_1 and F_2 progenies of three crosses, Apex, Thatcher and Red Egyptian respectively with P.W. 357-5, were studied to investigate the inheritance of stem rust resistance in the seedling stage.

A large scale inoculation method to induce stem rust epidemics suitable for greenhouse conditions was devised and described. This method is not only suitable for genetical studies but may be useful for purifying breeding stocks.

Apex and Chinese were susceptible in the seedling stage to the stem rust races used (mainly race 56) while Thatcher, Red Egyptian and P.W. 357-5 were highly resistant.

Resistance to stem rust contributed by P.W. 357-5 was dominant in the F_1 progenies of the three crosses.

In the cross Apex × P.W. 357-5 the observed results indicated that a 27 : 37 ratio was involved. The chi-square test for goodness of fit resulted in a P value between 0.90 and 0.95. It was concluded that three complementary factor pairs governing the resistance to stem rust are present in the perennial wheat.

In the cross of Thatcher × P.W. 357-5, a theoretical ratio was calculated on the assumption that three complementary dominant genes for resistance are present in P.W. 357-5 and that three recessive genes for resistance are present in Thatcher. The good fit obtained was considered as further evidence that P.W. 357-5 carries three pairs of dominant complementary genes for resistance.

In the cross of Red Egyptian × P.W. 357-5 an hypothesis was developed and tested that besides the two parental types of resistance, resistance was obtained from the complementary action of r_1 with any one of P_1, P_2 and P3, and also from the complementary action of r_2 with P_1P_2. The satisfactory fit obtained strengthened the evidence that P.W. 357-5 has three complementary dominant genes for resistance in the seedling stage to some of the existing races of stem rust.

Since the variety Chinese is susceptible to the race of stem rust used, then the resistance in the perennial wheat most likely had its source from the grass *A. elongatum*.

Note:

Since this paper was written a sister line, S-44-2-7, of P.W. 357-5 has been reported to be resistant to stem rust race 15B. Since all the progeny of the F_4 plant from which S-44-2-7 and P.W. 357-5 were developed have been resistant in the rust nursery at Saskatoon, the authors feel confident that the mode of inheritance of resistance reported above also applies to race 15B.

The resistance of S-44-2-7 to race 15B as reported by C. O. Johnson appears in the report of the Wheat Stem Rust Conference, University Farm, St. Paul, Minnesota, November 17–18, 1950. (*Mimeographed.*)

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APPENDIX A

TABLE OF GENOTYPES FOR FIVE PAIR FACTORS

AA = P_1P_1 BB = P_2P_2 CC = P_3P_3
dd = r_1r_1 ee = r_2r_2 R = Res. S = Sus.

(1)

1	AABBCCDDEE
2	AABBCCDDEe
2	AABBCCDdEE
2	AABBCcDDEE
2	AABbCCDDEE
2	AaBBCCDDEE
4	AABBCCDdEe
4	AABBCcDDEe
4	AABbCCDDEe
4	AaBBCCDDEe
4	AABBCcDdEE
4	AABbCCDdEE
4	AaBBCCDdEE
4	AABbCcDDEE
4	AaBBCcDDEE
4	AaBbCCDDEE
8	AABBCcDdEe
8	AABbCCDdEe
8	AaBBCCDdEe
8	AABbCcDDEe
8	AaBBCcDDEe
8	AaBbCCDDEe
8	AABbCcDdEE
8	AaBBCcDdEE
8	AaBbCCDdEE
8	AaBbCcDDEE
16	AABbCcDdEe
16	AaBBCcDdEe
16	AaBbCCDdEe
16	AaBbCcDDEe
16	AaBbCcDdEE
32	AaBbCcDdEe
243	R (ABC)

(2)

1	AABBCCDDee
2	AABBCCDdee
2	AABBCcDDee
2	AABbCCDDee
2	AaBBCCDDee
4	AABBCcDdee
4	AABbCCDdee
4	AaBBCCDdee
4	AABbCcDDee
4	AaBBCcDDee
4	AaBbCCDDee
8	AABbCcDdee
8	AaBBCcDdee
8	AaBbCCDdee
8	AaBbCcDDee
16	AaBbCcDdee
81	R (ABC)

(3)

1	AABBCCddEE
2	AABBCCddEe
2	AABBCcddEE
2	AABbCCddEE
2	AaBBCCddEE
4	AABBCcddEe
4	AABbCCddEe
4	AaBBCCddEe
8	AABbCcddEE
8	AaBBCcddEE
8	AaBbCCddEE
16	AABbCcddEe
16	AaBBCcddEe
16	AaBbCCddEe
16	AaBbCcddEE
32	AaBbCcddEe
81	R (ABC)

(4)

1	AABBccDDEE
2	AABBccDDEe
2	AABBccDdEE
2	AABbccDDEE
2	AaBBccDDEE
4	AABBccDdEe
4	AABbccDDEe
4	AaBBccDDEe
4	AABbccDdEE
4	AaBBccDdEE
4	AaBbccDDEE
8	AABbccDdEe
8	AaBBccDdEe
8	AaBbccDDEe
8	AaBbccDdEE
16	AaBbccDdEe
81	(72R : 9 S)

(5)

1	AAbbCCDDEE
2	AAbbCCDDEe
2	AAbbCCDdEE
2	AAbbCcDDEE
2	AabbCCDDEE
4	AAbbCCDdEe
4	AAbbCcDDEe
4	AabbCCDDEe
4	AAbbCcDdEE
4	AabbCCDdEE
4	AabbCcDDEE
8	AAbbCcDdEe
8	AabbCCDdEe
8	AabbCcDDEe
8	AabbCcDdEE
16	AabbCcDdEe
81	(54 R : 27 S)

(6)

1	aaBBCCDDEE
2	aaBBCCDDEe
2	aaBBCCDdEE
2	aaBBCcDDEE
2	aaBbCCDDEE
4	aaBBCCDdEe
4	aaBBCcDDEe
4	aaBbCCDDEe
4	aaBBCcDdEE
4	aaBbCCDdEE
4	aaBbCcDDEE
8	aaBBCcDdEe
8	aaBbCCDdEe
8	aaBbCcDDEe
8	aaBbCcDdEE
16	aaBbCcDdEe
81	(54 R : 27 S)

(7)

1	AABBCCddee
2	AABBCcddee
2	AABbCCddee
2	AaBBCCddee
4	AABbCcddee
4	AaBBCcddee
4	AaBbCCddee
8	AaBbCcddee
27	R (ABC)

(8)

1	AABBccDDee
2	AABBccDdee
2	AABbccDDee
2	AaBBccDDee
4	AABbccDdee
4	AaBBccDdee
4	AaBbccDDee
8	AaBbccDdee
27	R (ee)

(9)

1	AAbbCCDDee
2	AAbbCCDdee
2	AAbbCcDDee
2	AabbCCDDee
4	AAbbCcDdee
4	AabbCCDdee
4	AabbCcDDee
8	AabbCcDdee
27	R (ee)

(10)

1	aaBBCCDDee
2	aaBBCCDdee
2	aaBBCcDDee
2	aaBbCCDDee
4	aaBBCcDdee
4	aaBbCCDdee
4	aaBbCcDDee
8	aaBbCcDdee
27	R (ee)

APPENDIX A—Continued

TABLE OF GENOTYPES FOR FIVE PAIR FACTORS—*Concluded*

AA = P_1P_1 BB = P_2P_2 C = P_3P_3
dd = r_1r_1 ee = r_2r_2 R = Res. S = Sus.

(11)

1	AABBccddEE
2	AABBccddEe
2	AABbccddEE
2	AaBBccddEE
4	AABbccddEe
4	AaBBccddEe
4	AaBbccddEE
8	AaBbccddEe
27	R (dd)

(12)

1	AAbbCCddEE
2	AAbbCCddEe
2	AAbbCcddEE
2	AabbCCddEE
4	AAbbCcddEe
4	AabbCCddEe
4	AabbCcddEE
8	AabbCcddEe
27	R (dd)

(13)

1	aaBBCCddEE
2	aaBBCCddEe
2	aaBBCcddEE
2	aaBbCCddEE
4	aaBBCcddEe
4	aaBbCCddEe
4	aaBbCcddEE
8	aaBbCcddEe
27	R (dd)

(14)

1	AAbbccDDEE
2	AAbbccDDEe
2	AAbbccDdEE
2	AabbccDDEE
4	AAbbccDdEe
4	AabbccDDEe
4	AabbccDdEE
8	AabbccDdEe
27	(18 R : 9 S)

(15)

1	aaBBccDDEE
2	aaBBccDDEe
2	aaBBccDdEE
2	aaBbccDDEE
4	aaBBccDdEe
4	aaBbccDDEe
4	aaBbccDdEE
8	aaBbccDdEe
27	(18 R : 9 S)

(16)

1	aabbCCDDEE
2	aabbCCDDEe
2	aabbCCDdEE
2	aabbCcDDEE
4	aabbCCDdEe
4	aabbCcDDEe
4	aabbCcDdEE
8	aabbCcDdEe
27	(18 R : 9 S)

(17)

1	AABBccddee
2	AABbccddee
2	AaBBccddee
4	AaBbccddee
9	R (dd)

(18)

1	AAbbCCddee
2	AAbbCcddee
2	AabbCCddee
4	AabbCcddee
9	R (dd)

(19)

1	aaBBCCddee
2	aaBBCcddee
2	aaBbCCddee
4	aaBbCcddee
9	R (dd)

(20)

1	AAbbccDDee
2	AAbbccDdee
2	AabbccDDee
4	AabbccDdee
9	R (ee)

(21)

1	aaBBccDDee
2	aaBBccDdee
2	aaBbccDDee
4	aaBbccDdee
9	R (ee)

(22)

1	aabbCCDDee
2	aabbCCDdee
2	aabbCcDDee
4	aabbCcDdee
9	R (ee)

(23)

1	AAbbccddEE
2	AAbbccddEe
2	AabbccddEE
4	AabbccddEe
9	R (dd)
2	aabbCcDDEE
4	aabbCCDdEe
4	aabbCcDDEe
4	aabbCcDdEE
8	aabbCcDdEe
27	(18 R : 9 S)

(17)

1	AABBccddee
2	AABbccddee
2	AaBBccddee
4	AaBbccddee
9	R (dd)

(18)

1	AAbbCCddee
2	AAbbCcddee
2	AabbCCddee
4	AabbCcddee
9	R (dd)

(19)

1	aaBBCCddee
2	aaBBCcddee
2	aaBbCCddee
4	aaBbCcddee
9	R (dd)

(20)

1	AAbbccDDee
2	AAbbccDdee
2	AabbccDDee
4	AabbccDdee
9	R (ee)

(21)

1	aaBBccDDee
2	aaBBccDdee
2	aaBbccDDee
4	aaBbccDdee
9	R (ee)

(22)

1	aabbCCDDee
2	aabbCCDdee
2	aabbCcDDee
4	aabbCcDdee
9	R (ee)

(23)

1	AAbbccddEE
2	AAbbccddEe
2	AabbccddEE
4	AabbccddEe
9	R (dd)

(24)

1	aaBBccddEE
2	aaBBccddEe
2	aaBbccddEE
4	aaBbccddEe
9	R (dd)

(25)

1	aabbCCddEE
2	aabbCCddEe
2	aabbCcddEE
4	aabbCcddEe
9	R (dd)

(26)

1	aabbccDDEE
2	aabbccDDEe
2	aabbccDdEE
4	aabbccDdEe
9	S

(27)

1	AAbbccddee
2	Aabbccddee
3	R (dd)

(28)

1	aaBBccddee
2	aaBbccddee
3	R (dd)

(29)

1	aabbCCddee
2	aabbCcddee
3	R (dd)

(30)

1	aabbccDDee
2	aabbccDdee
3	R (ee)

(31)

1	aabbccddEE
2	aabbccddEe
3	R (dd)

(32)

1	aabbccddee
1	R (dd)

APPENDIX B

Calculation for five pair factors in 1024 plants

(A)

$P_1P_2P_3$ (ABC)

(1)	243
(2)	81
(3)	81
(7) +	27
	432 R

(B)

r_1r_1 (dd)

(11)	27
(12)	27
(13)	27
(17)	9
(18)	9
(19)	9
(23)	9
(24)	9
(25)	9
(27)	3
(28)	3
(29)	3
(31)	3
(32) +	1
	148 R

(C)

r_2r_2 (ee)

(8)	27
(8)	27
(10)	27
(20)	9
(21)	9
(22)	9
(30) +	3
	111 R

(D)

r_1 with P_1, P_2 or P_3
(d with A, B or C)

(4)	54
(5)	54
(6)	54
(14)	18
(15)	18
(16) +	18
	216 R

(E)

r_2 with P_1P_2
(e with AB)

(4)	18 R

Total Resistant Plants

(A)	432
(B)	148
(C)	111
(D)	216
(E) +	18
	925 R

1024 − 925 = 99 S

Theoretical Ratio = 925 R : 99 S for 1024 plants

or

1067 : 114 for 1181 plants

THE CONSERVATION OF SOIL MOISTURE IN SOUTHERN SASKATCHEWAN[1]

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INTRODUCTION

Summerfallowing to control weeds and to conserve soil moisture under dry-land conditions is a necessary though rather inefficient practice. Under the conditions which prevail in southern Saskatchewan, the moisture stored in the soil during the summerfallow period is usually only 15 to 30 per cent of the precipitation received. During summer the losses are large, due to evaporation and, sometimes, run-off. In winter, the snow is held fairly well on stubble fields but frequently blows off bare summerfallow. Much of the accumulated snow may be lost as run-off if a rapid thaw occurs in the spring. The loss from evaporation is usually small in winter, although it may be appreciable when chinook winds occur.

Under semi-arid conditions very little moisture is lost below the root zone; hence the moisture economy can be studied readily by soil moisture sampling. Experiments conducted on field strips at Swift Current (11) showed that 24 per cent of the precipitation was conserved during the summerfallow period. As much as 62 per cent of the total storage was conserved during the first fall and winter. The results obtained at experiment stations in the northern part of the United States apply, to some extent, to the conditions in southern Saskatchewan. Cole and Mathews (4, 5, 6) reported on the storage and use of water by spring wheat in co-operative projects throughout the Great Plains. They emphasized the high correlation between the stored moisture in the soil at seeding time and the resulting crop yields. Bell (2) discussed moisture conservation in summerfallow in north-central Montana. His data show that the moisture stored during the first fall and winter comprised about 60 per cent of the total. Thysell (14) at Mandan, North Dakota, reported that 20 per cent of a total precipitation of 22 inches was conserved during the summerfallow period. About 45 per cent was conserved during the first fall and winter and the remainder in the summer of the following year. A small loss occurred during the final winter. Bracken and Cardon (3) at Nephi, Utah, showed that 30 per cent of a total precipitation of 20 inches was conserved in summerfallow. Fairly large gains were made during the winter, but the soil lost moisture during the dry summer months.

This paper shows the amount of moisture stored during different divisions of the 21-month summerfallow period under practical farming conditions in southern Saskatchewan.

EXPERIMENTAL

Moisture conservation was obtained from soil samples taken on 10 Experimental Substations operated under the supervision of the Experimental Farms system. Most of the stations were within a 100-mile radius

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of Swift Current. Two were located on clay soil and the remainder were on loam. Data are available from 1937 to the present, except for 1943.

Two sampling sites on each station were selected on adjoining strips of a 2-year rotation of wheat and summerfallow. No attempt was made to take samples throughout the fields because variation in soil texture and land level would make the number of samples required prohibitive. Samples were taken from nearly level, well-drained rectangular areas, 10 by 20 yards, on each strip, these sites remaining unchanged as far as possible year after year. Six locations were sampled in each rectangle, viz., at the corners and the mid-points of the sides. Five samples, representing depths 0–6, 6–12, 12–24, 24–36 and 36–48 inches, were taken at each location. Volume weights of the soils were obtained for converting percentage moisture to inches of available water. The volume weight procedure consisted of weighing cores of soil, 2 inches in diameter and 2 inches high, taken at different depths along the side of a trench.

Samples for moisture determination were taken twice a year on the strips being fallowed—in the spring usually during the first week of May, and at freeze-up around the end of October. Samples were taken on the cropped strips at seeding time, at harvest and again at freeze-up, the actual dates of sampling varying somewhat depending on the season. The 5 sets of samples per year were sufficient to give fairly complete information on the 21-month summerfallow cycle on both strips. Moisture conservation was obtained for the 4 periods: harvest of the crop year to freeze-up; freeze-up to early spring; spring to freeze-up of the fallow year, and freeze-up to seeding time of the next crop.

The over-all conservation was based on the soil moisture content at harvest. Sometimes this was a little above the permanent wilting percentage of the soil and sometimes below, depending on whether or not rains had occurred before the sampling date. The mean conservation for a number of years should be a good measure of the water stored and used in the rotation. Weed growth reduced the moisture stored in some cases although this loss on substations should be less than the average for the area. Trash covers have been maintained on some fallow fields in recent years. Run-off would be comparable to that on farms with gentle slopes.

RESULTS

Seven years' measurements of moisture conservation in summerfallow are shown in Table 1. The table includes the amount of moisture conserved, the precipitation, and the percentage of the precipitation conserved during the 4 main divisions of the summerfallow period. The mean precipitation for the whole 21 months was about 19 inches. Of this a mean of 2.2 inches was received during the first 3 months, from harvest until freeze-up. The mean winter precipitation (November to April) was approximately twice that of the fall period, and it occurred twice in the summerfallow cycle. The mean summer precipitation (May to October) was 7.8 inches, three to four times that received in the fall months and almost twice that received from November to April.

The mean conservation for the whole period from the harvest of one crop to the seeding of the next was 4.0 inches or 21 per cent of the precipitation. The percentage conservation was generally quite high during

TABLE 1.—MOISTURE CONSERVED IN SUBSTATION SOILS DURING 21-MONTH SUMMERFALLOW PERIODS

21-month fallow periods	No. of stations	Stubble						Fallowed						21-month total		
		August to October			November to April			May to October			November to April					
		Cons.	Prec.	Cons.	Cons.	Prec.	Cons.	Cons.	Prec.	Cons.	Cons.	Prec.	Cons.	Cons.	Prec.	Cons
		in.	in.	%	in.	in.	%	in.	in.	%	in.	in.	%	in.	in.	%
1939–40–41	6	0.5	1.2	42	1.7	4.9	35	2.0	8.0	25	0.9	3.7	24	5.1	17.8	29
1940–41–42	10	1.3	2.3	57	0.7	4.0	17	0.7	8.0	9	1.9	4.5	42	4.6	18.8	24
1944–45–46	7	0.4	1.6	25	2.2	4.6	48	1.2	7.2	17	−0.5	3.4	—	3.3	16.8	20
1945–46–47	7	1.8	3.1	58	0.7	3.1	23	1.5	9.2	16	0.1	5.2	2	4.1	20.6	20
1946–47–48	7	1.2	3.1	39	1.3	5.0	25	0.9	9.4	10	1.5	5.5	27	4.9	23.0	21
1947–48–49	6	1.0	3.2	31	2.2	5.7	39	−0.2	5.3	—	0.2	2.8	7	3.2	17.0	19
1948–49–50	6	−0.3	0.7	—	0.7	2.8	25	1.2	7.8	15	1.1	5.6	20	2.7	16.9	16
Mean		0.8	2.2	36	1.4	4.3	33	1.0	7.8	13	0.7	4.4	16	4.0	18.7	21

the first fall and winter when the fields were still in stubble, and low in the summer and the following winter when they were in bare fallow. A mean of 0.8 inch was conserved from harvest to freeze-up in the pre-fallow year and 1.4 inches over the first winter. The 2.2 inches of moisture stored in these two periods was 33 per cent of the precipitation.

From May to October the mean gain was only 1.0 inch or 13 per cent of a rainfall of nearly 8 inches. The following over-winter period was scarcely more efficient with 0.7 inch conserved or 16 per cent of a precipitation of 4.4 inches. This analysis shows that a little over half of the moisture stored by the summerfallow, 2.2 inches out of 4.0 inches, was already in the soil before any work had been done on the land.

The values quoted above are the average of 7 years only, and probably the results from a longer period would be somewhat different. However, the data for the different periods are similar to those obtained in long-time experiments at the Swift Current station. The amount and percentage of conservation vary widely from year to year, indicating that a number of conflicting factors interact to produce the net gain in any one season. The yearly totals in the right-hand side of Table 1 differ sufficiently to produce large differences in the subsequent crop yields.

Conservation from August to October in Stubble Fields

It is not surprising that moisture conservation is high in stubble land when rains occur around harvest time. A crop dries the soil to the wilting point or lower, thus leaving it in an ideal condition for absorbing water. Some of the moisture from even light showers will penetrate to a sufficient depth to be safe from evaporation. Losses from run-off are not usually serious because of the soil conditions and because sudden heavy showers are infrequent after harvest. The chief loss during the period, apart from evaporation, is due to the growth of weeds. The effect of fall cultivation to prevent such losses is being tested at the Swift Current Experimental Station. The Noble blade, which leaves the stubble standing, and the one-way disk are used. The data in Table 1 show that cultivation in a dry fall such as 1948 would be unprofitable since a mean of only 0.7 inch of precipitation was received. Cultivation usually lowers the height of the stubble and possible losses from reduced snow accumulation must be weighed against gains from the removal of weeds. In Montana fall disking of stubble decreased the yields of summerfallow wheat (2) and fall tillage with blade-type implements was not found beneficial (1).

Conservation from November to April in Stubble and Fallow Fields

The mean conservation of winter precipitation was 1.4 inches in stubble fields and 0.7 inch in summerfallow. The difference between stubble and fallow was partly due to the difference in depth of snow accumulation on the two surfaces. The winter precipitations shown in columns 7 and 13 of Table I differ slightly because the same substations were not used in each summerfallow cycle. The conservation in stubble was consistently higher than in fallow even though the values from year to year varied widely. The gains for the individual stations contributing to the yearly means differed also, but they showed a definite correlation, indicating that certain weather conditions were common to all the stations in any one

year. The negative conservation in summerfallow in 1944–46 was due to the loss of surface moisture—the 0·6 inch layer was moist in the fall and dry in the spring. The benefit from snow was appreciable in stubble in all years whereas it was of value in fallow about 50 per cent of the time.

The precipitation for the period November to April was mostly snow received while the soil was frozen. However, some rain and snow occurred in November before freeze-up and a greater amount came early in the spring after the soil had thawed out. In late fall and early spring the rate of evaporation is low relative to that in summer so that, unless chinook winds occur, a high percentage of the precipitation received during these periods is stored in the soil.

The efficiency of the midwinter snowfall is reduced by losses from snow drifting, run-off and evaporation. Table 2, made up of data from plots and fields at Swift Current, shows the magnitude of some of the factors affecting the absorption of water from snow. Columns 2 and 3 give the available soil moisture to a depth of 4 feet in stubble and fallow fields at freeze-up. By available moisture is meant the moisture that can be used by crops or the amount of moisture in excess of the permanent wilting percentage. Columns 4 and 5 show respectively the precipitation for the whole period November to April, and the precipitation received while the soil was unfrozen. The latter comprised from one-quarter to over one-half of the total precipitation. The snow depths in columns 6 and 7 are approximate since, in some years, the snow almost disappeared a number of times before the final March thaw. In contrast with these data, during recent winters when the snowfall was heavy and thaws infrequent, snow depths of 8 to 18 inches on stubble and 6 to 13 inches on summerfallow were observed. The density of snow in March was such that about 3 inches of snow produced 1 inch of water. Except for the occasional year of good snow coverage the summerfallow became bare after the first day or two of thaw. The resulting conservations in columns 8 and 10 show more gain in stubble and less in fallow than was obtained during recent years on the substations. Reference to the data in columns 4 and 5 shows that high conservation occurred on years of high precipitation, and that much of the conservation must be attributed to the precipitation received when the soil was not frozen. However, late fall and early spring precipitation could not account for all the storage in stubble fields. Even in fallow some moisture is conserved when a dry fall is followed by a winter of good snow coverage.

The second last column of Table 2 gives the dates on which the rapid spring thaws began. In most cases 4 or 5 clear days, with air temperature of 40° F. or over, and only moderate freezing at night, were sufficient to produce a rapid run-off, and to remove most of the normal snow accumulation. In years such as 1940 two dates were recorded since the thaw was halted by a period of cold weather. In other years frequent thaws throughout the winter kept the snow accumulation low, and no definite date could be established. The last column gives the approximate dates on which the soil temperatures at a depth of 1 foot were raised to 32° F. Soil temperatures taken in stubble and fallow plots during this period showed that the stubble reached 32° F. a few days before the fallow. Where two dates were recorded, the first is for the stubble and the second for the summerfallow. Comparison of the last two columns of the table shows that the

TABLE 2.—CONSERVATION OF WINTER PRECIPITATION IN STUBBLE AND FALLOW FIELDS AT SWIFT CURRENT 1936–1944

Season	Available moisture at freeze-up		Precipitation November to April		Snow depth in early March		Moisture conserved over winter				Date of rapid thaw	Soil temperature above 32° F. at 1-foot depth
	Stubble	Fallow	Total	Precipitation while soil unfrozen	Stubble	Fallow	Stubble		Fallow			
	in.	in.	in.	in.	in.	in.	in.	%	in.	%		
1936–37	0.1	1.5	2.0	0.5	5	1	0.8	40	−0.1	—	Mar. 4	Apr. 9
1937–38	0	1.4	7.3	3.6	7	5	4.7	64	1.5	21	" 11	Mar. 19
1938–39	1.2	5.0	3.9	1.1	4	0–3	1.7	44	−0.7	—	" 18	" 27
1939–40	0	4.0	3.9	1.9	—	—	2.4	62	0.9	23	" 1 and 14	April 17
1940–41	0.7	4.3	2.9	0.8	0	0	0.3	12	0.4	14	Open winter	Mar. 24
1941–42	0.5	1.5	5.1	3.4	5–6	0–4	2.5	49	1.1	22	Mar. 4	" 11 and 18
1943–44	2.5	4.7	2.7	1.1	—	—	0.9	33	−0.2	—	Open winter	April 4 and 9
Mean	0.7	3.2	4.0	1.8	—	—	1.9	47	0.4	10	— —	— —

soil at 1-foot depth was still frozen when rapid thaw and run-off occurred. The lag of 8 and 9 days in 1938 and 1939 is what might be expected following continued warm weather. In other years colder weather intervened and the soil did not thaw out for a month or so after the winter's snow had disappeared. Frost limited the permeability even though an appreciable amount of moisture had entered the surface soil by the time the frost was out at the 1-foot depth. The efficiency of penetration was variable, depending on snow cover, rate of thaw and soil conditions. In the spring of 1937 the soil became waterlogged to a depth of 5 inches on the first day of rapid thaw. In 1938, the soil was thawed to a depth of 1 or 2 inches when the run-off reached its peak; in 1939, ice was forming at the soil surface when run-off occurred. Although the loss is often high, the evidence shows that winter precipitation is beneficial on stubble fields in most years. Good weed control the year round is likely to contribute to moisture conservation during the winter period. A weed-free crop leaves a tall, clean stubble which is ideal for holding snow. Field observations in the early spring indicate that the density of snow in stubble is less when the latter is filled with a thick growth of Russian thistle. The net effect may be contributed to by the fact that the dark-coloured thistles absorb more radiation than the stubble, thereby inducing rapid thawing and evaporation.

The moisture conserved over winter in summerfallow is often negligible. The greatest accumulation in fallow occurred in years when the snow, whether from midwinter or late spring storms, formed a hard crust and remained in drifts covering the fields. Trash covers are of some benefit in holding a cover and in initiating the formation of drifts. Preliminary experiments with snow ploughing at the Scott Experimental Station (9) did not result in appreciable increases in the yield of cereal crops, although hay crops benefited consistently.

Conservation from May to October in Summerfallow

The largest loss during the May to October period is due to evaporation. Staple and Lehane (12) have shown that satisfactory estimates of evaporation can be made using meteorological data. In order to add to the stored soil moisture, the precipitation must be sufficient to saturate the dry surface soil and cause water to move downward beyond a depth of 4 or 5 inches. A shower which does not wet the surface sufficiently to make contact with the moist soil below is soon lost by evaporation. Thus the moisture storage depends largely on the characteristics of the rainfall itself. The evaporation loss is less in the cooler months such as May and October than in July and August. To conserve moisture the individual showers must either be heavy enough to penetrate below the evaporation zone, or occur at close enough intervals that their cumulative effect will produce the same result.

The 22-year mean moisture storage for May to October on loam soil at the Swift Current station was 16 per cent of the rainfall. It was estimated that rainfall penetrated the dry surface soil on the average only seven times during the 6-month period. The maximum number of effective showers was 18 in 1939, and the minimum 0 in 1937. Following a long dry period, a rainfall of 0.75 inch would be required before any water would penetrate deeply enough to be safe from evaporation. On the other hand, as in 1939, rainfalls of as little as 0.1 or 0.2 inch were effective when received on consecutive days.

The mean percentage conservation for the 7 years shown in Table 1 was only 13 per cent. This value was affected considerably by weed growth, even though the weed control on the substations was better than average. Losses due to run-off, which are appreciable in some years, were minimized by the choice of sampling sites. The loss of moisture below a depth of 4 feet was negligible for the periods studied.

The possible effects of cultivation, apart from that designed for weed control, should be mentioned. The dust mulch has long been discarded as a method of conserving moisture under dry-land conditions. A moderately lumpy surface is recommended to prevent soil drifting and to permit rapid percolation in the case of heavy showers. Experience has shown that the nature of the cultivation can differ widely without having much effect on moisture conservation. If the soil becomes too hard or too finely pulverized there may be serious loss from run-off during thunder-showers. On the other hand, a soil that is extremely loose and lumpy will dry out rapidly and reduce the efficiency of the rainfall.

Cultural experiments at Swift Current (11) (13) showed that various implements, such as the one-way disk, cultivator and plough used alone or in various combinations conserved almost equal amounts of soil moisture provided the weed growth was kept down. From northern Montana, Bell (2) reported that ploughed and ploughless fallow had almost identical soil moisture reserves at seeding time but that the average yield of spring wheat was slightly higher when the plough was used. Ploughing after every third or fourth crop was recommended. Aasheim (1) showed that no great differences in soil moisture, wheat yields or quality of wheat were produced by the use of different combinations of cultural implements including ploughs, cultivators, blade weeders, etc., during the fallow period. Englehorn (7) in North Dakota found that tillage with the field cultivator and stubble mulch tillage yielded as well as ploughed fallow.

The trash cover or straw mulch, recommended for control of wind and water erosion, plays only a minor role in reducing evaporation during the May-to-October summerfallow period. Measurements at Swift Current have shown that the additional moisture conserved with a trash cover (1 to 2 tons per acre) in summer is of the order of 0.25 inch. Appreciable gains would be expected only in the case of a high water table, or when—as sometimes occurs in the spring or following heavy rains—the subsoil moisture remains at or above the field capacity for an extended period. The effectiveness of the straw mulch should be examined more closely in the early spring, and in more humid districts, to find the maximum possible conservation.

Weed growth is still the main factor that the farmer can control in the conservation and retention of soil moisture in summerfallow. Cultivation should be commenced early enough to prevent spring weeds from sapping the 2.2 inches of moisture concentrated near the surface from the previous fall and winter. The moisture content of the surface 4 or 5 inches of soil determines the effectiveneess of a given rainfall—the higher the surface moisture, the less the rainfall required to reach below the evaporation zone. A young growth of weeds feeding on the surface moisture will reduce the penetration of later rains occurring in May or early June.

The spring treatment of summerfallow is sometimes difficult, following a series of dry years, because of the likelihood of soil drifting. Low precipitation results in short crops with little stubble to hold snow or to form a trash cover. The soil moisture may be almost nil in the spring, and early cultivation of summerfallow may be unjustified if the soil is likely to drift. Such fields are often left unworked until rains of an inch or more are received and some moisture conservation seems worth attempting.

The greatest loss is sustained in seasons when a good gain in soil moisture is made during the first fall and winter, but cultivation of summerfallow is not commenced until late June or July. A heavy growth of weeds removes the stored moisture, and only small gains are made during the remainder of the fallow period. In other cases the growth is kept down in the spring, but a few scattered weeds are permitted to grow to maturity in the late fall, the hope being that the protection from wind and the moisture from additional snow accumulation will more than offset the loss of stored moisture. This is a hazardous procedure since the moisture losses due to even thin stands of weeds are large. In addition, the conservation from snow is uncertain and the fields become infested with weeds. The maintenance of a good trash cover to prevent drifting and hold some additional snow would be preferable.

An extreme example of the above is the uncultivated "summerfallow" used in certain districts where sandy loam soils are susceptible to soil drifting. There can be no doubt that the weeds take all the moisture conserved during the previous fall and winter, and greatly increase the weed-seed population. That good yields are sometimes obtained from this practice is probably due to the nature of the weed growth in these districts. A tall, open stand of weeds that matures early will, with suitable precipitation, conserve moisture through the fall period, and hold a depth of snow in winter equal to twice that on a normal stubble field. Such a weedy summerfallow fails completely where the weed growth is a short, thick mat of Russian thistles. In any case, the practice is likely to increase the infestation of both annual and perennial weeds. A better solution should be sought in some form of crop rotation so that paying crops might replace the weeds. The objective should be, as in all good farming, to reduce the growth of weeds in the crop, thereby producing a better stand of grain and leaving a cleaner, taller stubble to conserve fall moisture and hold snow for the next year's crop.

MOISTURE CONSERVATION AND DEPTH OF MOIST SOIL

It is of interest to consider what the moisture storage in Table 1 represents in terms of depth of moist soil. Table 3 gives average data for different soil classes. The field capacities were obtained by a modification of the method of Olmstead (10), and the wilting percentages by the method described by Work and Lewis (15). The field capacity is the amount of water held by the soil a few days after a heavy rainfall when downward movement of moisture has almost ceased. The permanent wilting percentage is the lower limit of soil moisture below which plant growth cannot be maintained. It is approximately equivalent to the moisture content of the soil at harvest time. The moisture held by the soil between the wilting percentage and the field capacity is available for growth of the crop. By

TABLE 3.—SOIL MOISTURE RELATIONSHIPS—AVERAGE FIELD CAPACITY, WILTING PERCENTAGE AND STORAGE CAPACITY OF SOILS

Soil textural class	Field capacity	Wilting percentage	Normal storage capacity to 4 feet
	%	%	in.
Sandy loam	10	3.5	4.0
Loam and silt loam	18	7.0	6.2
Clay loam and silty clay loam	26	10.5	7.2
Clay	35	17.0	8.6

seeding time in southern Saskatchewan the soil moisture content at plough depth is usually a little below field capacity. The best estimate of the maximum available moisture stored in the soil at seeding time, expressed as inches of water held by a 4-foot depth of soil, is given in column 4 of Table 3 under the heading, "Normal storage capacity". According to these data, 2.2 inches of water stored in loam from harvest until spring would moisten a depth of 17 inches to normal capacity. The depth of moist soil in stubble fields at seeding time is clearly defined since the moisture content changes abruptly from near field capacity to wilting percentage. The depth of moist soil converted by the use of data in Table 3 is then a fairly accurate measure of the available water.

The situation is different in summerfallow since a slow downward movement takes place during the long summerfallow period. Instead of a sharp break from very moist to dry soil, the moisture content tapers gradually and the exact depth is not readily measured. Also, because much of the soil is appreciably below the field capacity, the depth of moist soil is not a reliable measure of the water content. For instance, the average of 4.0 inches of water conserved in summerfallow (Table 1) would represent a depth of 31 inches moistened to near field capacity. The mean depth of moist soil in the springtime is greater than this because the moisture content at the lower depths is usually well below the field capacity.

In recent years (6) (8) farmers have been advised to measure the depth of moist soil in stubble and summerfallow fields at seeding time. The soil is considered moist if it sticks together when pressed into a ball in the hands. The depth of moisture in loam soil at seeding time varies in stubble fields from 12 to 30 inches and in summerfallow from 25 to 50 inches. The depth of spring moisture tells a farmer something of his crop prospects, and how much rain will be needed to give an average yield. In some cases testing for moisture will help him to decide which fields should be seeded. Care should be used in interpreting the depth of moist soil found in summerfallow. This method of measurement is not accurate enough to establish small differences in moisture conservation.

STORED MOISTURE AND SUBSEQUENT CROP YIELD

Sampling for soil moisture and crop yield has been carried out on substations in the Swift Current area each year since 1937 except for 1943 (11). In analysing these data the water used by the crop was assumed to be that removed from the soil plus the rainfall received during the crop season.

The results showed that 5 or 6 inches of water were required, on the average, to produce a minimum yield of one or two bushels of wheat per acre. With more available water the efficiency increased slowly until 10.5 inches of water produced a mean yield of 14 bushels per acre. For higher yields the increase in yield with each additional inch of water was almost constant at 6 bushels per acre. This applies, of course, only to the range of moisture and yield found in the Swift Current area. The yield must become independent of soil moisture under more humid conditions when water is no longer a limiting factor. The relationship between water use and yield described above is but the general trend in data which show considerable scatter. Differences in rainfall distribution, evaporation, weed growth, etc., change the effectiveness of moisture use from year to year.

The mean moisture used from the soil by summerfallow crops on the substations during an 11-year period was 4.3 inches and the seasonal precipitation was 6.6 inches. In the average season then, the rainfall will provide the moisture required (5 to 6 inches) for the minimum vegetation growth of the crop, but without the moisture stored in the soil, the grain yields on many years would be nil.

SUMMARY

Seven years' measurements of moisture conservation on the substations in southwestern Saskatchewan are presented.

A mean precipitation of 18.7 inches received during the 21-month summerfallow period was made up as follows: August to October, 2.2 inches; November to April, 4.3 inches; May to October, 7.8 inches and November to April, 4.4 inches.

The average moisture conserved in stubble fields at seeding time was 2.2 inches and in summerfallow 4.0 inches. The mean conservation for the first 9 months was 33 per cent of the precipitation, for the last 12 months it was 14 per cent, and for the whole period it was 21 per cent.

The winter precipitation, which was largely snowfall, was consistently beneficial in stubble fields. It provided two-thirds of the moisture stored in stubble at seed-time.

The moisture conserved over winter in bare summerfallowed fields was often negligible. Trash covers were of some benefit in holding a snow cover, preventing soil drifting and reducing evaporation.

During the summer months showers must be fairly large (0.4 to 0.8 inches), or occur at frequent intervals in order to penetrate below the evaporation zone.

Weed growth causes large losses of soil moisture during the summerfallow period. Cultivation of summerfallow should be commenced early enough in the spring to prevent loss of moisture stored over the fall and winter.

ACKNOWLEDGMENT

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RUTIN CONTENT OF VARIETIES OF BUCKWHEAT[1]

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Rutin, a flavonol glucoside compound, was discovered over a century ago in the garden rue, *Ruta graveolens*, whence its name. It remained a laboratory curiosity until 1943, when Couch *et al.* (3) established its use to restore increased capillary fragility and permeability in man. Further extensive clinical uses have since been reported.

Rutin is widespread in nature, having been reported in 40 species. The first clinical experiments used rutin extracted from the tobacco plant. Buckwheat received attention in 1946 when Couch, Naghski and Krewsen (1) reported that of all species examined buckwheat proved to be the most promising and economical source. In that year rutin was produced commercially in Canada from Japanese buckwheat (*Fagopyrum esculentum*). Experimental evidence in 1949 (2) indicated that Rye buckwheat (*F. tartaricum*) was superior to the Japanese variety previously used.

EXPERIMENTAL MATERIAL

During the 4 years, 1947-1950, twenty-four varieties and strains of buckwheat were tested for rutin content.

The experiment included both the *tartaricum* and *esculentum* species. The fertility of the two species differs in that *F. tartaricum* has been found to be mainly self-fertile while *F. esculentum* is highly self-sterile. For that reason the plant improvement procedure followed with the *esculentum* species has consisted of covering the progeny of a parent in muslin cages.

The varieties of the *esculentum* group include two varietal types, Silverhull and Japanese. The Japanese type is characterized by larger, darker coloured seed, and a more vigorous growth than the Silverhull type. Four selections of Silverhull were tested and included with this group was an introduction from Portugal designated Portuguese in this paper. The Japanese type consisted of Japanese, Common Black, and a group of introductions from Russia listed under the introduction numbers of C.D. 1356, 1361, 1370 and 1374. Two others, C.D. 5043 and C.D. 5044, received from Sweden were tested in 1950 only. The latter is a tetraploid variety.

The *tartaricum* group included four varieties—Welsford, Tartarian, Rough and C.D. 4251. Welsford is a vigorous growing variety extensively grown in New Brunswick. It grows about 4 inches taller than other varieties in this group and produces higher yields. C.D. 4251 is an introduction from the United States which was obtained from a Canadian company producing rutin commercially.

The above varieties were grown on clay loam soil in 1947 and 1950 and on sandy loam during 1948 and 1949. Each year the test was seeded around the middle of June and samples were harvested when blossoming first commenced which was usually 35 to 40 days after seeding.

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CHEMICAL ANALYSIS OF BUCKWHEAT FOR RUTIN

Representative samples of 20, 25 or 30 plants, usually 25, were taken from the sheaf harvested. The number of plants was chosen for each variety so as to give a sample of between 150 and 250 gm. Duplicate fresh samples were extracted by the method of the Eastern Regional Research Laboratory, U.S. Department of Agriculture (4) using 600 ml. alcohol for 4 hours and 400 ml. alcohol for the remainder of 24 hours. The method was modified by the introduction of an extraction with petroleum ether prior to the evaporation of alcohol. The alcohol solution from the buckwheat extraction was extracted in a separatory funnel with petroleum ether, enough water being added to give two distinct layers and the petroleum ether discarded. It was found that no rutin was removed by the petroleum ether and this removal of impurities greatly facilitated the separation of the rutin and also increased the purity.

The same number of plants as used for the fresh sample was selected for drying. In this procedure an attempt was made to simulate commercial practice. The sample was cut in pieces 1 to 2 inches long and dried in a "moisture teller" at 110° C. for 10 minutes, stirred and dried an additional five minutes. The leaves and blossoms became dry and brittle and readily separated from the soft wet stems. Leaves and blossoms were redried for one minute to remove any moisture acquired from contact with the stems. The dried sample was analysed by the method (4) of the Eastern Regional Research Laboratory for dried material. In all cases the rutin content on the dried material was somewhat lower than on the fresh.

The data given in Table 1 were from the entire plant freshly harvested.

TABLE 1.—PER CENT RUTIN ON A DRY MATTER BASIS IN BUCKWHEAT VARIETIES GROWN AT THE CENTRAL EXPERIMENTAL FARM, 1947-1950

Variety	1947	1948	1949	1950	Average 1948-50
Japanese—B & O	—	—	3.44	3.67	—
Japanese—Virg.	—	2.16	3.70	3.33	3.06
Japanese—40–2	5.9	2.40	4.06	3.46	3.31
C.D. 1356—40–3	4.4	2.96	3.71	2.82	3.16
C.D. 1356—42–3	—	2.31	3.71	2.76	2.93
C.D. 1356— –7	4.8	2.31	4.09	4.35	3.58
C.D. 1356— –8	4.6	—	3.19	3.88	—
C.D. 1356— –14	5.8	4.16	2.86	2.21	3.08
C.D. 1361—11	4.0	2.07	4.60	2.97	3.21
C.D. 1370— 7	4.6	3.15	4.49	4.48	4.04
C.D. 1374— 4	6.5	—	3.66	5.10	—
C.D. 1374— 5	5.0	2.24	3.43	2.60	2.76
C.D. 5043	—	—	—	3.92	—
C.D. 5044	—	—	—	3.63	—
Common Black	4.8	2.85	3.41	3.48	3.25
Silverhull—3	—	2.59	4.17	3.95	3.57
Silverhull—4	—	3.29	4.35	4.98	4.21
Silverhull—7	—	3.42	3.34	4.89	3.88
Silverhull—10	—	3.48	3.88	3.66	3.67
Portuguese	—	3.33	3.04	3.28	3.22
Welsford	—	2.93	2.85	4.69	3.49
Tartarian	—	2.98	4.59	3.60	3.72
Rough 4	—	2.73	3.74	3.66	3.38
C.D. 4251	—	—	4.65	5.71	—
Mean	5.04	2.85	3.77	3.80	—

In 1947, ten varieties only were tested. The results disclosed a very encouraging rutin content which varied from 4.0 to 6.5 per cent. The average of over 5 per cent for this year was the highest of the 4 years in which tests were made. During 1948, the rutin contents for seventeen varieties were low, ranging from 2.16 per cent to 4.16 per cent with a mean of 3.85 per cent. In the following two years (1949-50) the analyses showed a rutin content averaging nearly 1 per cent higher. The analysis of variance for the three years (1948-50) on seventeen varieties is given in Table 2.

TABLE 2.—ANALYSIS OF VARIANCE ON PER CENT RUTIN (1948-50)

Source of variation	Degrees of freedom	Sum. of squares	Mean squares
Total	50	2831.16	—
Years	2	758.95	379.47*
Varieties	16	723.03	45.19
Error	32	1349.18	42.17

* Significant at 1 per cent level.

From the above table it will be noted that varietal differences are not significant while the various years significantly affected the rutin content. Such a conclusion is difficult to explain since the results do not follow the seasonal variations. It will be noted from Table 3 that 1948 and 1949 were similar with respect to rainfall and temperature and both were abnormal. On the other hand 1950, like 1947, was cool and very wet, particularly throughout the critical month of July. The season of 1950 appeared to favour the varieties Welsford and Silverhull. The Japanese type appeared to be influenced more by temperature than by the wide variations prevailing in moisture conditions during these years.

TABLE 3.—METEOROLOGICAL DATA FOR JULY AT THE CENTRAL EXPERIMENTAL FARM, OTTAWA, ONTARIO, 1947-1950

Year	Av. max. temp.	Av. min. temp.	Mean temp.	Rainfall, inches	Number of days with precipitation	Av. sunshine per day
1947	78.1	60.7	69.4	5.15	13	6.7
1948	80.3	58.0	69.1	2.97	12	9.7
1949	83.8	59.0	71.5	2.78	6	10.0
1950	78.3	58.3	68.3	5.74	13	8.1

Under the conditions of these tests the *tartaricum* group did not differ significantly from the *esculentum* group. On the average, the Silverhull type appears to be a little higher in rutin content than Japanese. Couch *et al.* (2) have pointed out that the *tartaricum* group have a higher proportion of leaves and yield a greater amount of leaf per acre. This would be especially true of the Welsford variety as it is a very vigorous growing variety. The tetraploid, although only tested for one year, did not appear more promising than the normal types.

SUMMARY

Tests for rutin content were made on twenty-four strains of buckwheat. The group included varieties of both the *tartaricum* and *esculentum* species.

No significant differences were found in per cent rutin between the different strains tested. Differences between years, however, were highly significant, the values ranging from 2.85 per cent in 1948 to 5.04 per cent in 1947.

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PARATHION RESIDUES ON SWEDE TURNIPS[1]

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Parathion has been shown to be a very effective insecticide for the control of turnip aphid (*Rhophalosiphum pseudobrassicae* (Davis)) which in 1949 was a most serious pest of turnips in Ontario. Swede turnips for table use are an important commercial crop in Central Ontario, and there is considerable export to the Eastern United States. In view of the highly toxic nature of parathion it was considered advisable to secure information on residues present at time of harvest.

Ginsberg *et al.* (3) analysed samples from two crops of turnips (Purple Top Globe) which were dusted with 1 per cent parathion. Both crops were dusted on September 1 and one crop was given a second dusting on September 10. Harvesting was 53 days after the first dusting and 44 days after the second. The accumulated rainfall was 4.48 inches. In both cases the peel showed a trace of parathion. The crop which had been dusted twice was tested for translocation, with a completely negative result.

EXPERIMENTAL

Parathion residue studies were conducted in 1949 and 1950 on Laurentian turnips. All plots were located on the Ontario Agricultural College farm. In both years randomized 1/40th acre plots were arranged in four blocks, the respective treatments being replicated in each block. All blocks contained a blank check and a water check, and were separated by a distance of 30 ft. In 1949 one series of four plots received a total of four weekly applications (August 10, 18 and 25, and September 3) of parathion at the commonly recommended rate of 1 lb. of 15 per cent wettable powder per acre, applied in 80 gal. of water, thus making a seasonal total of 0.6 lb. of actual parathion per acre. A single plot was given two very heavy applications (August 25 and September 3), each consisting of 10 lb. of 15 per cent wettable powder per acre. This represents a seasonal total of 3 lb. actual parathion per acre.

In 1950 one series of four plots was sprayed with 15 per cent wettable parathion at the rate of 1 lb. per acre and a second series of four plots at 5 lb. per acre. The plots in blocks I and III received four sprays at approximately fortnightly intervals—July 15 and 29, and August 15 and 28. These plots were sampled 8 and 14 days following the last spray. Root samples and some foliage samples were taken, the latter since turnip leaves are sometimes fed to livestock after harvest. The plots in blocks II and IV received only three sprays, the last of the above sprays being omitted. They were sampled 27 days after the last spray.

In sampling, a representative number of turnips was pulled at random from each plot, the edges of the plots being avoided to prevent border effect. In 1949 each sample was composed of 25 to 30 turnips; but in 1950, owing to the larger size of the turnips, only about one-half that number constituted the sample.

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Loosely adhering soil was removed from the fresh plant material by gently brushing, and extraneous roots were trimmed off. The turnips were then peeled and extracted at once. 500 gm. samples of peel and of foliage and 700 gm. samples of pulp were taken for extraction. Peel and pulp samples were ground to a smooth puree in a Waring Blendor, a portion of the benzene used for extraction being added at this time to aid in the maceration. Tinfoil-lined screw caps were used on the glass containers of the Blendor. The samples were then extracted with reagent grade benzene in an end-over-end mechanical shaker revolving at a rate of about 75 r.p.m. The extraction period was 40 minutes in 1949 and 75 minutes in 1950.

The extracts were analysed for parathion by the method of Averell and Norris (1). Colorimetric measurements were made with an Evelyn photoelectric colorimeter using filter No. 540.

Since the turnip extracts were quite highly coloured a preliminary decolourization was necessary. In 1949 the decolourizing mixture recommended by Averell and Norris (1) was used, but in 1950 the U.S. Food and Drug Administration decolourizing mixture (3) was adopted, since it was found to be superior to the former for the decolourization of turnip extracts.

Some trouble was experienced in the final stages of the determination due to the occasional formation of an opalescent precipitate upon dilution of the alcohol-water solution containing the reduced parathion with water or with reagents (such as sodium nitrite and ammonium sulphamate) in water solution. Apparently this is due to alcohol-soluble substances which have been extracted by the benzene. Norris (5) has found that in most cases this difficulty can be eliminated by diluting the alcoholic solution to about 35 ml. with water and allowing it to gradually cool to room temperature before filtering off the excess zinc dust and precipitated plant substances. This modification proved to be very helpful in overcoming our trouble. Gunther and Blinn (4) have found that these substances appear to be completely soluble in 60 per cent alcohol, and have accordingly revised the procedure, adding 50 ml. more of ethyl alcohol and finally diluting to 100 ml. instead of 50 ml.

Analyses were made on samples from the control plots and corrections have been applied to all data reported. While members of the family Cruciferae (such as Brussels sprouts, broccoli, and cabbage) frequently give high blanks (1), there was no particular difficulty of this nature in our study.

RESULTS AND DISCUSSION

Table I shows the results of the parathion determination.

The 1949 results showed no evidence of translocation of parathion, even in the sample from the plot which was given two very heavy applications. Ginsberg *et al.* (3), as mentioned above, also found no evidence for translocation in turnips. Since it appeared obvious that translocation does not occur, pulp determinations were not made in 1950. The 1950 residue values for "entire root" in Table I are computed values based on the assumption that no translocation occurred.

It may be observed that the ratio between the respective "peel" and "entire root" residues is much narrower in 1949 than in 1950. This is explained by the fact that the turnips sampled in 1949 were considerably

TABLE I.—RESIDUES RECOVERED FROM TURNIPS (LAURENTIAN) SPRAYED WITH PARATHION

Sample No.	Description	Treatments Acre Basis 15% Wettable Powder	Dates Parathion Applied	Dates Parathion Harvested	Days After Last Treatment	Accumulated Rainfall Inches	Residue P.P.M.	Residue Based on Entire Root P.P.M.
			1949 RESULTS					
1 (a)	Peel	1 lb.	8/10, 8/18,	9/12	9	2.28	.21	.038
(b)	Pulp	1 lb.	8/25, 9/3,	9/12	9	2.28	.00	
2 (a)	Peel	10 lb.	8/25, 9/3,	9/12	9	1.46	1.21	.252
(b)	Pulp	10 lb.	8/25, 9/3,	9/12	9	1.46	.00	
			1950 RESULTS					
3	Foliage, Blocks I and III	1 lb.	7/15, 7/29,	9/5	8	7.77	.02	
4	Foliage, " "	5 lb.	8/15, 8/28,	9/5	8	7.77	.21	
5	Peel, " "	1 lb.	As in No. 3&4	9/5	8	7.77	.10	.005
6	Peel, " "	5 lb.	" "	9/5	8	7.77	.21	.013
7	Peel, " "	1 lb.	" "	9/11	14	7.77	.02	.001
8	Peel, " "	5 lb.	" "	9/11	14	7.77	.15	.009
9	Foliage, Blocks II and IV	5 lb.	7/15, 7/29, 8/15	"	27	7.77	.01	
10	Peel, " "	1 lb.	" " "	"	27	7.77	Trace (.004)	Trace
11	Peel, " "	5 lb.	" " "	"	27	7.77	.10	.007

smaller than those sampled in 1950, the latter being of normal marketable size at time of sampling. In 1949 the peel averaged 18 per cent of the entire turnip, whereas in 1950 it averaged only 6 per cent.

It will be observed that residues on comparable samples tended to be somewhat higher in 1949 than in 1950. While most of the loss of parathion after application to plants is usually attributable to its volatility, plant growth and weathering are also factors in the reduction of residues. In 1949 the four applications of insecticide on th series of randomized plots were made over a period of approximately $3\frac{1}{2}$ weeks, whereas in 1950 the same number of applications extended over a period of slightly more than 7 weeks. Thus, due to the increased period of time over which the applications were made in 1950, decreased residues would be expected. The much higher rainfall during the experimental period in 1950 is doubtless another factor. However, while the rainfall recorded for this period in 1950 was 7.77 inches, the last two insecticidal applications were exposed to only 2.52 inches, the only heavy rainfall recorded during August being 1.22 inches on the 29th. In 1949 the last three insecticidal applications received only 1.46 inches, the only appreciable rainfall again having occurred on August 29—0.72 inches. The more rapid growth of the more mature turnips in 1950 would also result in lower parathion residue values, since these are expressed on a weight basis.

The residues on peel and foliage are quite low even for those samples which had received much heavier applications than usually recommended. Furthermore, results are for unwashed turnips. Washing the turnips would no doubt have yielded still lower residues on peel. The common practice of peeling the turnips, in their preparation for the table, should result in practically no parathion being present in the portion consumed. The amounts of residual parathion which would have been ingested, if the turnip leaves sampled in this experiment had been fed to dairy cows in maximum quantities, are very much smaller than the amounts of parathion fed by Dahm *et al.* (2) without observing any harmful effects to the health of the cows, or detecting any parathion in the milk.

SUMMARY

In this experiment parathion residues on turnips at time of harvest were determined. Parathion had been applied to these turnips at the rate commonly recommended for the control of turnip aphid (1 lb. of 15 per cent wettable powder per acre) and at heavier rates, every week or two for a period of 2 to 8 weeks. Samples were taken, 1, 2, and 4 weeks after the last insecticidal application, and foliage, peel, and pulp analyses were made.

Surprisingly low residues were found on peel and leaves and none in pulp, even when abnormally heavy applications of the insecticide had been made. In 1949 the application of four weekly sprays at the rate of 1 lb. of 15 per cent wettable powder per acre resulted in a residue on peel of 0.21 p.p.m., 9 days after the last treatment. In 1950 a similar application, but made bi-weekly, resulted in a residue of 0.10 p.p.m. on peel and 0.02 p.p.m. on foliage, 8 days after the last treatment. When 5 lb. of wettable powder were used per treatment the corresponding residues on peel and foliage were both 0.21 p.p.m. In 1949 two 10-lb. applications of 15 per cent

wettable powder 9 days apart resulted in a residue of 1.21 p.p.m. 9 days after the second treatment. Residues decreased quite rapidly with increase of time following applications.

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